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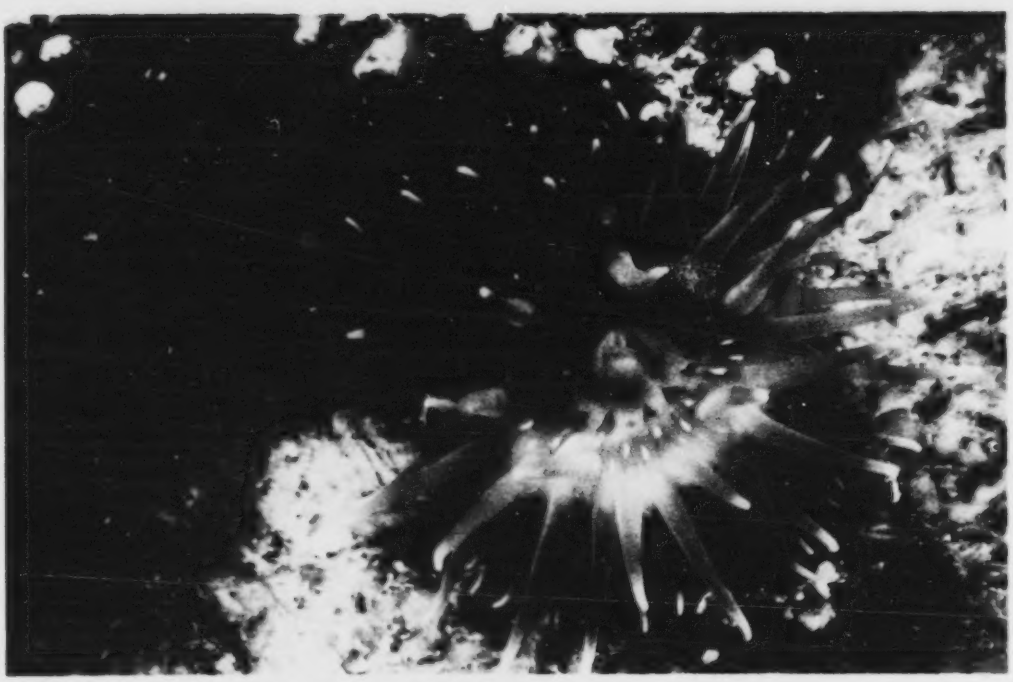
APPLICATION OF MICROBIAL EXOENZYME ACTIVITY MEASUREMENTS TO ASSESS THE IMPACT OF DREDGE SPOILS DISPOSAL IN PICTOU HARBOUR, NOVA SCOTIA AND THE MIRAMICHI RIVER, NEW BRUNSWICK
OCEAN DUMPING REPORT 6

K. Lee, K.L.Tay, E.M. Levy, C.N. Ewing and S.E. Cobanli



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APPLICATION OF MICROBIAL EXOENZYME ACTIVITY MEASUREMENTS TO ASSESS THE IMPACT OF DREDGE SPOILS DISPOSAL IN PICTOU HARBOUR, NOVA SCOTIA AND THE MIRAMICHI RIVER, NEW BRUNSWICK

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ABSTRACT

Lee, K., K.L. Tay, E.M. Levy, C.N. Ewing and S.E. Cobanli. 1990. Application of microbial exoenzyme activity measurements to assess the impact of dredge spoils disposal in Pictou Harbour, Nova Scotia and the Miramichi River, New Brunswick. Ocean Dumping Report 6, Environment Canada: iii + 95 p.

An environmental impact assessment procedure based on the measurement of microbial exoenzyme activity in sediments was evaluated at sites in Pictou Harbour, Nova Scotia and the Miramichi River, New Brunswick. Both had received contaminated dredge spoils. Activity of the exoenzymes glucosidase, phosphatase and protease was variable with no spatial correlation to designated dumpsite locations. Chemical analyses of corresponding sediments also failed to delineate dumpsite locations. Results of field trials to date, substantiate the appropriateness of microbial exoenzyme measurements for evaluating condition and recovery of sediment disposal areas in a rapid and cost-effective manner.

RÉSUMÉ

Lee, K., K.L. Tay, E.M. Levy, C.N. Ewing et S.E. Cobanli, 1990. Utilisation de mesures d'activité d'exoenzymes microbiens pour l'évaluation de l'impact de l'immersion de déchets de dragage dans le havre de Pictou en Nouvelle-Écosse et dans la rivière Miramichi au Nouveau-Brunswick. Immersion de déchets en mer, rapport 6, Environnement Canada: III + 95 p.

Une procédure d'évaluation d'impact environnemental basée sur des mesures de l'activité des exoenzymes microbiens dans les sédiments a été évaluée dans le havre de Pictou en Nouvelle-Écosse et dans la rivière Miramichi au Nouveau-Brunswick. Chaque site avait reçu des déchets de dragage contaminés. L'activité des exoenzymes glucosidase, phosphatase et protéase était variable et ne montrait aucune corrélation spatiale avec la location des sites d'immersion de déchets. Les analyses chimiques des sédiments correspondants n'ont pas permis non plus de délimiter les sites d'immersion de déchets. Les résultats d'essais sur le terrain appuient jusqu'à présent l'utilisation des mesures d'activité d'exoenzymes microbiens pour l'évaluation des conditions et du rétablissement des zones d'immersion de sédiments d'une façon rapide et économique.

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1.0 INTRODUCTION

Marine sediments are the primary sites of such environmentally vital processes as organic matter degradation, nutrient regeneration and trace metal transformation (Degobbis et al., 1986; Lee et al., 1985; Haines et al., 1982). Since these processes are mediated predominately by the activity of sediment microorganisms, assessment of sediment microbial activity should be included as an essential part of any environmental impact assessment.

Previous studies of the effects of sediment disposal operations on microbiota have tended to focus on changes in oxygen demand (Phoel et al., 1981) or introduction of foreign bacteria, especially fecal coliforms from sewage sludge disposal (Graikoski, 1981). While perturbations on such processes tend to be short-lived (for example, Phoel et al. (1981) reported that oxygen consumption rates returned to background levels in as little as four days following a disposal event), there is no doubt that other microbial processes can be adversely affected for extended periods by contaminants. For example, Griffiths et al. (1982a, 1982b) reported that exposure to crude oil caused immediate cessation of nitrogen fixation in sediments, a reduction in the rates of organic matter degrading enzymes (chitobiase, cellulase, laminarinase and phosphatase) and significant reductions in nitrogen cycling processes for more than 18 months.

Lee et al. (1990b) proposed that long-term environmental impacts at dredge spoil disposal sites could be assessed by comparing microbial exoenzyme activity in sediments impacted by sediment disposal operations with those found in adjacent, non-impacted areas, even if pre-disposal enzyme activity rates were not known. With support from the Environment Canada, Ocean Dumping Research Fund, procedures to measure bacterial exoenzyme activity in sediments were improved and used for environmental impact assessment at the Heron Island ocean dumpsite near Dalhousie, New Brunswick.

Exoenzymes, which by definition are those located outside the cytoplasmic membrane (Priest, 1984) are involved in polymer degradation and have been found dissolved in the water or adsorbed to particles. The penetration of organic molecules across a bacterial cell wall is an active process occurring with the intervention of specific enzymes called permeases. Only small organic molecules (monomers or small polymers) can be taken up in this manner. Since a major fraction of the dissolved organic matter (DOM) in the aquatic environment consists of compounds with high molecular weight (Allen, 1986), such as proteins, polysaccharides, nucleic acids and humic materials (Munster, 1985; Stabel et al., 1979), they cannot be assimilated by bacteria directly. Through the production of exoenzymes induced by the presence of suitable substrate, and after subsequent hydrolysis of compounds to monomers or oligomers, most DOM can be ultimately absorbed by bacteria (Rogers, 1961).

Although disposal activities at the Heron Island ocean dumpsite have ceased ten years prior to the survey, the enzyme assay technique showed significant depression of exoenzyme (cellulase, phosphatase and protease) activity at the dumpsite center and positive correlations between enzyme activity with increased distance from the dumpsite (Lee et al., 1990B). The present study details results of additional field trials with this assessment procedure.

The activity of specific inducible exoenzymes in natural waters can be measured through the use of nonfluorescent substrates conjugated with the compound, methylumbelliferone (MUF) (Hoppe, 1983; Meyer-Reil, 1986; Lee et al., 1987). The high fluorescence of methylumbelliferone which is released following hydrolysis of specific substrates allows the measurement of enzymatic activity with short incubation times, at ambient temperatures, and at substrate concentrations similar to those found in nature. However, since it is not known to what extent MUF substrates represent naturally occurring substances in seawater, or whether the specificity of the enzyme acting on the substrate has a broad or narrow spectrum, it is recommended that activity be measured for a number of enzyme systems, to ascertain the condition of such a diverse community as sediment bacteria. In the present study, fluorogenic substrate specific for glucosidase (4-methylumbelliferyl glucoside), phosphatase (4-methylumbelliferyl phosphate) and protease (L-Leucine-4-methyl-

coumarinyl-7-amide HCl) are used to evaluate the impact of disposed sediments on biogeochemical processes at dumpsites located in Pictou Harbour, N.S. and the Miramichi River, N.B.

In 1987, during the development of the Pictou Industries Wharf, 195,000 m^3 of predominantly organic clay sediments were deposited at two dumpsites in a deep channel in the center of Pictou Harbour. Dredge sediments removed from the inner harbour were contaminated primarily by effluent from a pulp mill and a variety of industrial wastes. To reduce the potential environmental impact of PCBs in the dredge spoils of up to 800 ng g^{-1} in some surface samples (OceanChem Group Ltd., 1987), silt curtains were deployed at both the dredge site and the dumpsites to minimize suspension and dispersal of the contaminated sediments (OceanChem Group Ltd., 1987). After completion of the dredging operation, dumpsite surfaces were smoothed to reduce irregularities.

Dredging of the shipping channel in the Miamichi River, N.B., in 1981 and 1982 by Public Works Canada generated 4.7×10^6 m^3 of sediments. These dredge sediments were deposited at three dumpsites: in the river between the cities of Newcastle and Chatham (Dumpsite A), and the estuary and bay at the river's mouth. "Contaminated" sediments (mostly silt and clay) from the inner shipping channel, containing high concentrations of wood fiber from pulp mills near Newcastle and elevated concentrations of heavy metals (lead, zinc and cadmium) from the local industrial sites, were deposited at Dumpsite A. To contain the wood fiber and heavy metals in these dredge spoils, a cap of uncontaminated sand was placed over the dumpsite (MacLaren Plansearch, 1985). "Clean" sediments from the middle and outer shipping channels were dumped at sites located in the estuary and bay.

2.0 MATERIALS AND METHODS

2.1 Field Sampling

Sediments were collected from Pictou Harbour on July 7, 1989 with the **M.V. Navicula** using a Ponar grab sampler (0.05 m^2). Samples along four transects were collected from 27 stations located both inside and outside dumpsite boundaries (Fig. 1, Appendix A4) previously defined by OceanChem Group Ltd. (1987).

Sediments were collected from the Miramichi River on July 9, 1989 with the **M.V. Navicula** using a Ponar grab sampler. Samples were collected at 23 stations along two transects passing through Dumpsite A located between Chatham and Newcastle, New Brunswick (MacLaren Plansearch, 1985). Stations were positioned both inside and outside the designated dumpsite boundaries (Fig. 2, Appendix A4).

Subsamples of approximately 50 cm^3 taken from the sediment grabs for measurement of exoenzyme activity (maintained at in situ temperature following collection) were assayed within 7 hours. Duplicate samples destined for chemical analysis were frozen in dry ice immediately after collection for transport to the laboratory.

2.2 Exoenzyme Activity Measurement

Using a modification of the procedure described by Hoppe (1983), specific enzyme substrates are used to measure the activity of microbial extracellular enzymes in sediments. Enzymatic hydrolysis of these nonfluorescent MUF substrate yields an equimolar amount of highly fluorescent 4-methylumbelliferone (MUF) and a substrate-dependant product. Exoenzyme activity in slurries of sediment suspended in seawater can thus be calculated from the amount of MUF liberated during the incubation period.

Methylumbelliferyl standards and exoenzyme substrate analogues were prepared from 4-methylumbelliferone (Sigma Chemical Co.), 4-methylumbelliferyl-B-D-glucoside (Sigma Chemical Co.), 4-methylumbelliferyl phosphate (Sigma Chemical Co.) or L-Leucine-4-methylcoumarinyl-7-amide HCl (Fluka Chemical Corp.), as outlined in Appendix A5.

Five replicate 0.2 cm^3 samples of homogeneously mixed sediment were dispensed into pre-weighed 7 mL polypropylene test tubes using small sterile core tubes (4 mm ID glass tube connected to a Pasteur pipette bulb). Three milliliters of 0.2 um filtered seawater were then added to each tube. The tubes were then capped with polyethylene stoppers, agitated and placed in an incubator, at in situ temperature. After 30 minutes of preincubation, 100 uL aliquots of specific enzyme substrate solutions (0.15 mM) were added to each tube (final substrate concentration of 5 uM). The assay tubes were incubated for 60 minutes, with agitation at 20 minute intervals. Immediately after this incubation period, the tubes were frozen in dry ice to arrest enzyme activity and transported to the laboratory for fluorescence analysis (Fig. 3). Duplicate blanks (SOW and sediment) and internal standards (1 uM MUF and sediment) were run concurrently.

The fluorescence of liberated methylumbelliferone (MUF) was measured using a Perkin-Elmer MPF-44B fluorescence spectrophotometer. Tubes were thawed in an ice bath and centrifuged at 1450 rpm for 5 minutes. The supernatant (2.5 mL) was decanted into a 1 cm path-length cuvette to which 0.25 mL of NaOH/Borax buffer (pH 10.8) was added to maximize MUF fluorescence. Fluorescence was measured at 455 nm emission with 365 nm excitation. The MUF concentration in each tube was calculated by comparison of the fluorescence to a standard curve prepared using known dilutions of a 1 uM solution of 4-methylumbelliferone.

Following fluorescence analysis, the dry weight of sediments in the experimental tubes was determined by drying the pre-weighed experimental tubes to constant weight in a convection oven (90°C). Exoenzyme activity was calculated as uM product/g sediment/h. Statistical analyses were made with the SYSTAT statistics computer software package (SYSTAT, 1987).

The detailed methodology for this assay is outlined under Appendices A5 and A6.

3.0 RESULTS

3.1 Pictou Harbour

While the lowest glucosidase activity values were found within the dumpsite boundaries (Stn 5, 12), significant differences in enzyme activity between stations inside and outside the designated dumpsite areas were not detected (Fig 4; Appendix A2, Table 1). Although there appeared to be increased activity toward the northeast (seaward) ends of both dumpsites, relative to the southwest ends (lowest at stns 5 & 12, increasing towards stns 8 & 15), high values were also observed at the SW ends (stns 3, 4 & 11). Further, there was no trend discerned on the transects running across the two dumpsites (Figs 5-6). Although phosphatase activity was approximately five-fold higher than that of glucosidase, observed trends in enzyme activity rates (Fig 7-9) were very similar (Appendix A2, Table 1). Except for two points, protease activity was very low (< 4 uM/g sediment/h) at the stations sampled (Appendix A2, Table 1), with no apparent trends in activity along either the SE-NW (Fig 10) or cross-wise transects (Fig 11-12). A Pearson correlation matrix of the physical, chemical and biological parameters measured at every station showed significant positive correlations ($p < 0.05$) between the activity of the three enzymes tested (Appendix A2, Table 2).

Results of chemical analyses conducted by OceanChem Group Limited, under contract to Environment Canada, are presented graphically for comparison with the exoenzyme assay results (Figs 13-20). Since chemical data were available for only 10 of the 26 stations sampled, at the time of this report's preparation, interpretation and statistical analyses were based on a relatively small data set ($n = 10$).

Concentrations of cadmium in the sediment samples analyzed were below the Canadian Environmental Protection Act (CEPA) limit of 0.6 mg kg^{-1} (Canada Gazette, 1988) and showed no apparent trend over the length of the SW-NE transect (Fig 13). While there was no definite trend in copper concentrations over the length of the transect, there appeared to be a slight reduction in concentrations towards the seaward (NE) end (Fig 14). Lead and

zinc concentrations on the SW-NE transect showed a reduction towards the seaward end of the western dumpsite, but not over the eastern dumpsite (Figs 15-16). The lowest zinc concentration was found outside the designated dumpsite boundary (stn 17), at the northeast end of the transect.

Like the metals Cd, Cu, and Zn, both total inorganic and total organic carbon appeared to be lower towards the seaward end of the SW-NE transect (Fig 17). PCB concentrations (CEPA regulatory limit: 100 ng g^{-1}) ranging from 28 to 101 ng g^{-1} were lower in the eastern dumpsite relative to the western dumpsite (Fig 18). With the exception of stn 1, along the transect from Stns 1 to 17, there appears to be an increase in PCB concentrations over the dumpsites (Fig 18). Concentrations of total oil and grease along the SW-NE transect were highly variable (Fig 19). Particle size analysis of sediments indicated a reduction in the percentage of fine material (silt and especially clay) towards the seaward end of the SW-NE transect (Fig 20).

A Pearson correlation matrix of the data from the stations where chemical parameters were measured showed that the enzyme activity was not significantly correlated ($P < 0.05$) to physical or chemical factors in the matrix (Appendix A2, Table 3). The PCB and oil/grease concentrations were not correlated with any of the parameters measured. Cadmium concentrations were positively correlated ($p < 0.05$) with organic carbon and total carbon and negatively correlated with the percent of sand in the sediment. Copper concentrations were positively correlated with inorganic and organic carbon concentrations and with the percentage of fine sediment particles (clay). Likewise, cadmium and copper concentrations were negatively correlated with the proportion of sand in the sediment.

Stepwise multiple regressions of the data showed that 77.5% of the observed variation in glucosidase activity could be explained by the variations in phosphatase activity. By including protease activity in the model, 87.9% of the glucosidase activity was explained (Appendix A2, Table 4). Phosphatase activity was best explained by glucosidase activity (77.5%) and the water depth (82.9%). Only glucosidase activity could significantly account for a proportion (72.1%) of the variation in protease activity.

3.2 Miramichi River

At Dumpsite A in the Miramichi River (Appendix A2, Table 5), significant differences in glucosidase activity were not observed between stations inside and outside the designated dumpsite boundary (Figs 21-22). There appeared to be a slight increase in glucosidase activity through the dumpsite on the SW-NE transect (Fig 21). However, this observation was not evident on the W-E transect where large variations in glucosidase activity were observed between adjacent stations, and higher enzyme activity rates were found outside the dumpsite boundary (Fig 22). Phosphatase activity was approximately two-fold higher than glucosidase activity and displayed the same trend of increased activity over the dumpsite on the SW-NE transect (Fig 23). As with glucosidase, there was no increase in activity over the dumpsite on the W-E transect, and activity was highest at the western end of the transect (Fig 24). There was no evidence of any trends in protease activity which showed large variations between adjacent stations over both transects surveyed (Figs 25-26). A Pearson correlation matrix of the data measured at every station showed a significant positive correlation ($p < 0.05$) between glucosidase and phosphatase activity (Appendix A2, Table 6). There were no correlations between enzyme activity and either water depth or distance from the dumpsite center.

Results of chemical analyses by OceanChem Group Limited on six sediment samples collected from the Miramichi River are listed in Appendix A3. Cadmium concentrations showed no trend along the transects through the dumpsite but were highest at the western end of the W-E transect (Figs 27-28). Except for one sample (station 14), all samples tested had cadmium concentrations below the CEPA regulated limit of 0.6 mg kg^{-1}. Copper concentrations in sediments from the dumpsite region were generally lower than those in surrounding sediments (Figs 29-30) and were highest at the west end of the W-E transect (stn 14). In our survey, the highest lead concentrations were observed at the west end of the W-E transect rather than in the vicinity of the dumpsite center as expected (Figs 31-32). Observed concentrations of zinc in the dumpsite sediments were slightly lower than in the sediments surrounding the dumpsite (Figs 33-34), partly due to the very high concentration at the west end of the W-E transect.

In general, the concentration of total carbon in sediments recovered from within the boundaries of the designated dumpsite was lower than in similar sediments from surrounding areas (Figs 35-36). This trend was pronounced for organic carbon. As was evident with the metals, the carbon concentration, especially organic carbon, was very high at the western end of the W-E transect (stn 14) but was also relatively high at the eastern end (stn 25). With the exception of station 17, PCB concentrations in dumpsite sediments were below the CEPA regulated limit of 100 ng g^{-1} (Figs 37-38). Oil and grease concentrations were generally lower in the sediments in the vicinity of the dumpsite than in the surrounding regions (Figs 39-40). Particle size analysis of sediments showed that the sediments from the dumpsite and station 11 on the SW-NE transect were composed almost entirely of sand; very little clay or silt was present (Fig 41). This distribution of sand over the dumpsite was also supported by the particulate analysis of samples from the W-E transect (Fig 42), which showed that sediments from both ends of the transect outside the dumpsite boundary were composed predominantly of fine material (silt and clay).

A Pearson correlation matrix of the measured chemical, physical and biological parameters showed that glucosidase activity had significant positive correlations ($p < 0.05$) with total inorganic carbon, total organic carbon, total carbon, cadmium, copper and zinc (Appendix A2, Table 7). Phosphatase and protease activity was not correlated with any of the measured physical or chemical parameters. PCB concentrations were significantly correlated with the proportion of gravel in the sediment. Oil and grease concentrations were positively correlated ($p < 0.05$) with both organic and inorganic carbon and fine sediment (clay and silt), and negatively correlated with the proportion of the sand in the sediments. The metals analyzed (Cd, Cu, Pb and Zn) were positively correlated with both organic and total carbon concentrations and the proportion of fine sediment (clay-sized particles). Copper and zinc concentrations were positively correlated with the concentration of silt in the sediment.

Stepwise multiple regressions of the data (Appendix A2, Table 8) indicated that the variations in glucosidase activity were best explained by the variations in phosphatase activity (80.1%). However, none of the measured parameters could be employed in a model that would explain the variations in protease activity.

4.0 DISCUSSION

4.1 Pictou Harbour

There is no evidence for a relationship between exoenzyme activity and the location of the sediment dumpsites in Pictou Harbour. Increased glucosidase and phosphatase activity towards the seaward ends of the dumpsites coincided with the lower concentrations of potential toxic metals. A positive correlation was found between enzyme activity and the concentration of metals (Cd, Cu, Zn and Pb). The significance of this correlation (Appendix A2, Table 3) is questionable due to the small size of the data set ($n = 10$). Variation in enzyme activity along the transects through the dumpsites appears to be correlated with sediment composition. The sediments at the seaward (NE) ends of both dumpsites exhibited greater proportions of sand, and lower proportions of silt and clay than sediments at the southwest ends of the dumpsites. Because this trend was more pronounced in the eastern dumpsite at the seaward end of the transect, it is possible that there was more erosion at the seaward ends of the dumpsites. The resulting loss of fine material could account for the reduced heavy metal concentrations at the seaward ends of the dumpsites, since metals tend to be associated primarily with the smallest sediment-size fractions and with the organic fraction of the sediment (Horowitz, 1985).

While the stepwise multiple regressions of the data showed that the activity of the individual enzymes could be best explained by other enzymes, not by any of the chemical factors, it seems plausible that variations in the activity of all three enzymes, in the complex chemical environment of Pictou Harbour, were the result of factors not measured or the interaction of measured parameters. Chemical parameters did not significantly contribute ($p < 0.05$) to the models explaining enzyme activity possibly because of limited availability of chemical data.

Exoenzyme activity measurements and chemical analyses suggest that microbial activity and chemical composition or both were not significantly different in sediments from the Pictou Harbour dumpsites and surrounding areas in the deep channel. It is hypothesized that the

dumpsites may have recovered to pre-disposal conditions with dispersal of the dredge spoils, by tide and storm action, over a much larger area than that defined by the original dumpsite boundaries. Rapid recovery to pre-disposal conditions is feasible given that these dumpsites were exposed to single disposal events of not extremely large volumes of dredge spoils.

4.2 Miramichi River

While some naturally occurring substrates have produced competitive inhibition of the enzymatic decomposition of MUF-phosphate, MUF-leucine, and MUF-glucopyranoside in seawater (Hoppe, 1983), sediments from the Miramichi River quenched the fluorescence of 4-methylumbelliferone considerably in the assay procedure. Fluorescence values were corrected for interference by using internal standards outlined in Appendix A6. Statistical analysis showed that exoenzyme activity in the Miramichi River sediments was not spatially correlated to dumpsite zones. In a report by MacLaren Plansearch (1985), the composition of sediments in the vicinity of the Miramichi River Dumpsite was described as being highly patchy in nature with a high degree of horizontal variability. This variability was also evident in the enzyme activity measurements made for this study. Exoenzyme activity was highest at the extreme west end of the W-E transect for all three enzymes assayed. The sediments in this area are characterized by elevated heavy metal concentrations, very high organic carbon concentrations and a high proportion of fine sediment particles. The high degree of correlation between glucosidase activity and chemical parameters suggests that the chemical and physical composition of the sediments in this area of the Miramichi River may affect microbial activity to a greater degree than the presence of the heavy metals in the dredge spoils alone. The absence of a corresponding correlation between phosphatase and protease activity with physical and chemical parameters is most likely the result of limitations in chemical data ($n = 6$).

Microbes may possess mechanisms for tolerating toxic metals (Gadd and Griffiths, 1978), and regions with high concentrations of organic carbon and a high proportion of fine sediment tend to promote bacterial activity (Lee et al., 1990b). Binding of metals to organic materials, precipitation, complexation, and ionic interactions alters their biological availability. In the natural environment, metals can be bound strongly by organic materials such as humic and fulvic acids and proteins (Benes et al., 1976; Stevenson, 1976). In aquatic environments, it has been demonstrated that metals such as zinc and copper can be bound and removed from the water by organic sediments, which effectively reduce the total metal ion concentration in solution (Bachmann, 1963; Whitton and Say, 1975). Clay particles can also reduce the toxicity of certain metals. Experiments with cadmium have shown that clay minerals, montmorillonite and kaolinite protected certain bacteria, actinomycetes, and filamentous fungi from the inhibitory effects of cadmium. The protective ability of the clays correlated with cation-exchange capacity (CEC); the greater the CEC, the greater the amount of cadmium absorbed (Babich and Stotzky, 1977).

MacLaren Plansearch (1985) reported that a cap of clean sand was placed over Dumpsite A in the Miramichi River to contain the dredged cadmium-contaminated sediments. In addition, a large proportion of the fine sediment fraction was lost from the dumpsite during and following disposal operations through resuspension and distribution downstream by the relatively high currents in the river. The high proportion of sand and low concentrations of potentially toxic metals in the dumpsite sediments, relative to surrounding areas, suggest that the sand cap is still in place. The low concentrations of metals, PCB's, carbon and oil in the dumpsite sediments most likely reflect the relatively uncontaminated nature of the sand used to cap the dumpsite. Chemical and physical analyses of the dumpsite sediments indicate that the grab sampler did not penetrate the cap, reported by MacLaren Plansearch (1985) to be at least 30 cm thick. The sediment at station 17 contained a high proportion of gravel and elevated PCB concentrations. This may represent an area where the cap had eroded. However, the low concentration of cadmium measured at this station (0.17 mg kg^{-1}) does not support this hypothesis.

4.3 Application of Enzyme Assay Procedures

Many waterways and harbours in industrial regions are dredged for navigation on a regular basis. Sediments from these regions, often contaminated, are a risk to biota during dredging and ocean-disposal operations. To accurately assess the environmental impact of a disposal event, it is necessary to use a battery of tests, since no single test will be adequate in all situations (Vives-Rego et al., 1986). Priority should be given to multispecies and system level tests in biological monitoring (Cairns, 1981). In aquatic systems, bacteria are particularly important because of their unique role in trophic dynamics. Bacteria provide a source of enriched particulate organic carbon through their efficient use of both dissolved and particulate carbon. Adverse influences on the composition or activity of this community could be detrimental to the aquatic ecosystem as a whole. Thus, if the full environmental impact of any perturbation on biological and chemical processes is to be known, the effects on the microbial community and its activity must be taken into consideration (Griffiths, 1983).

Microbial activity tests can be used to assess the effects of dredging or disposal activities on environmentally crucial processes. Environmental impact assessment procedures carried out in unenclosed natural systems are the most realistic since few extrapolations are needed. Subtle effects and interactions not present or easily detected in simplified systems can often be detected in field experiments, although they may be masked by high variability. Even if pre-disposal microbial activity is unknown, comparison of microbial community activity in the impacted area with activity in unimpacted but similar adjacent areas can yield meaningful results. This method of comparing microbial activity in an impacted dumpsite with activity in surrounding areas has been used successfully by Environment Canada to assess recovery of the Heron Island dumpsite (Bay of Chaleur, N.B.) several years after the cessation of disposal activities (Lee et al., 1990b).

Bacterial enzymes acting on 4-methylumbelliferyl (MUF) compounds have been used in clinical enzyme assays (Trepeta and Edberg, 1984) to identify fecal coliforms (Berg and Fiksdal, 1988), and to measure exoenzymatic activity in natural waters (Hoppe et al., 1988, Hoppe, 1983). Bacterial enzymes acting on MUF compounds in natural water are described as extracellular (Hoppe, 1983) and are at least partially cell bound, since enzymatic activity was retained on the filter after filtration of cell suspensions through a 0.22 um pore-size filter. In the present study, comparison of exoenzyme activity (measured with MUF compounds) within the designated dumpsites with activity in surrounding areas did not yield a clear indication of impact by dredge spoil disposal. This was likely due to the quick recovery of the dumpsites in Pictou Harbour, the stability of the sand cap over Miramichi River dumpsite and the possible development of tolerance by the microfauna to the contaminants. In support of the exoenzyme results, corresponding chemical analyses also failed to show the presence of contaminated dredge spoils. Similar studies at the Heron Island dumpsite, showed significant depression of enzyme activity and elevated metal concentrations at the dumpsite center (Lee et al. 1990b). Differences in the results observed are most likely due to the history of the dumpsites concerned (Appendix A2, Table 9). For many years, prior to its closure in 1978, the Heron Island dumpsite received a large volume of anoxic harbour sediments which were contaminated with wood fibers, lead, zinc and cadmium (Packman et al., 1984). In Pictou Harbour, the dumpsites received a relatively small volume of PCB contaminated dredge spoils during a single dredging operation (OceanChem Group Ltd.). While dumpsite A in the Miramichi River received a large volume of contaminated dredge spoils over a two year period, the dumpsite was capped with clean sand (MacLaren Plansearch, 1985). Both the Pictou Harbour and Miramichi River dumpsites are situated in relatively shallow river estuaries where they would be exposed to the eroding forces of tide and river currents. This would tend to lead to a more rapid recovery due to the dispersion of the finer sediment particles from the dumpsite with which the bulk of contaminants are associated. In contrast, the Heron Island dumpsite is situated in the deep basin of a large ocean bay where there would be relatively little erosion and, as shown by both enzyme activity and chemical analyses, a much slower recovery.

The 1988/1989 Environment Canada Ocean Dumping Research Fund supported the development of a bacterial exoenzyme assay procedure for environmental impact and dumpsite recovery assessment. This assay was to be based on the measurement of changes in the exoenzyme activity (a primary metabolic process found in microorganisms) of indigenous biota under conditions similar to that found in the environment in response to contaminants. The procedure is relatively rapid, inexpensive and simple enough to be performed by most consulting analytical laboratories with existing instrumentation. Further, to avoid interference from natural chemical reactions, and to increase reliability and reproducibility, routine internal standards are incorporated into the methodology. The assay was found to be highly sensitive to environmental contaminants (Lee et al., 1990a; Lee et al., 1987), and was subsequently incorporated into existing environmental impact and dumpsite recovery assessment programs.

Field trials with the exoenzyme assay demonstrated the long term impact of dredge spoils disposal at the Heron Island ocean dumpsite (Lee et al., 1990b). In the present study conducted at ocean dumpsites in Pictou Harbour and the Miramichi River, the exoenzyme assay procedure exhibited the dispersal of dredge spoils from the dumpsites in Pictou Harbour and the success of the capping operation at Dumpsite A in the Miramichi River. At all three study sites, a close correlation between the exoenzyme assays and physical/chemical analyses was evident. Due to the ecological significance of microbial processes, we recommend that microbial enzyme activity analyses be conducted as an integral part of environmental impact assessment programs.

5.0 ACKNOWLEDGEMENTS

This research program was funded by Environment Canada under the Ocean Dumping Research Fund. The authors wish to thank the Captain and crew of the **M.V. Navicula** for their assistance in the field, and B.L. Wilkinson for review of the manuscript.

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7.0 APPENDICES

A1. FIGURES

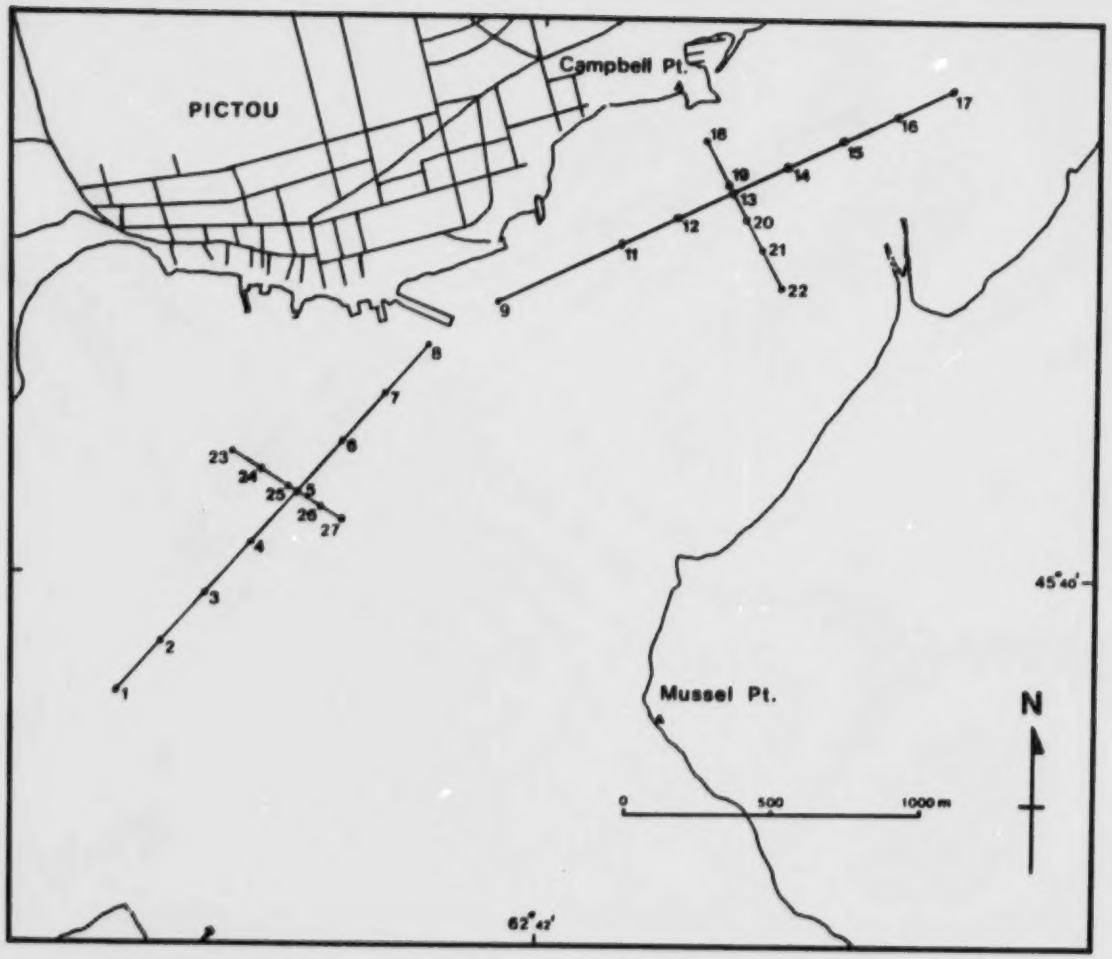


Figure 1: Sampling stations and locations of sediment dumpsites (shaded areas) in Pictou Harbour, Nova Scotia.

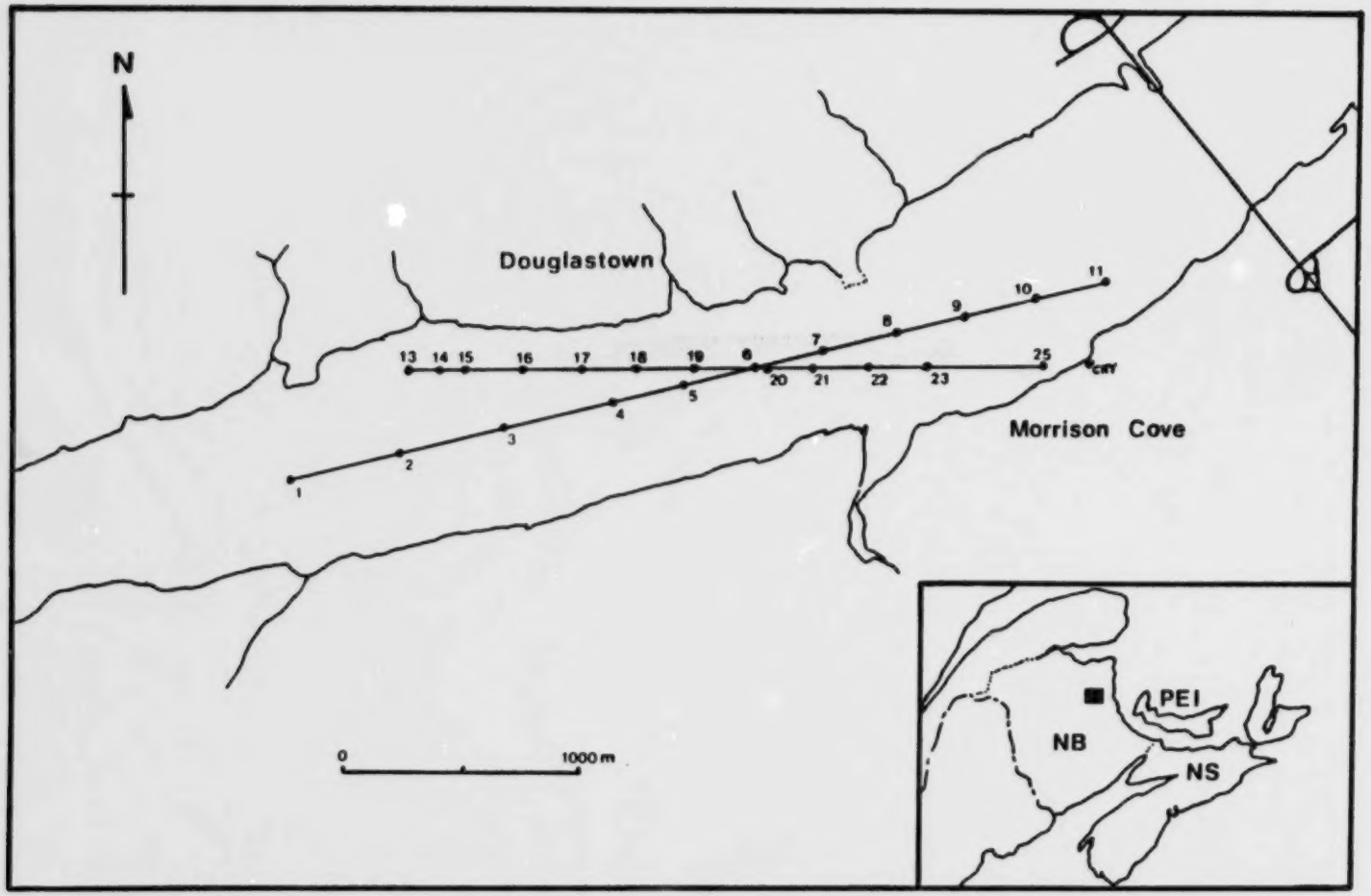


Figure 2: Sampling stations and location of sediment dumpsite "A" (shaded area) in the Miramichi River, New Brunswick.

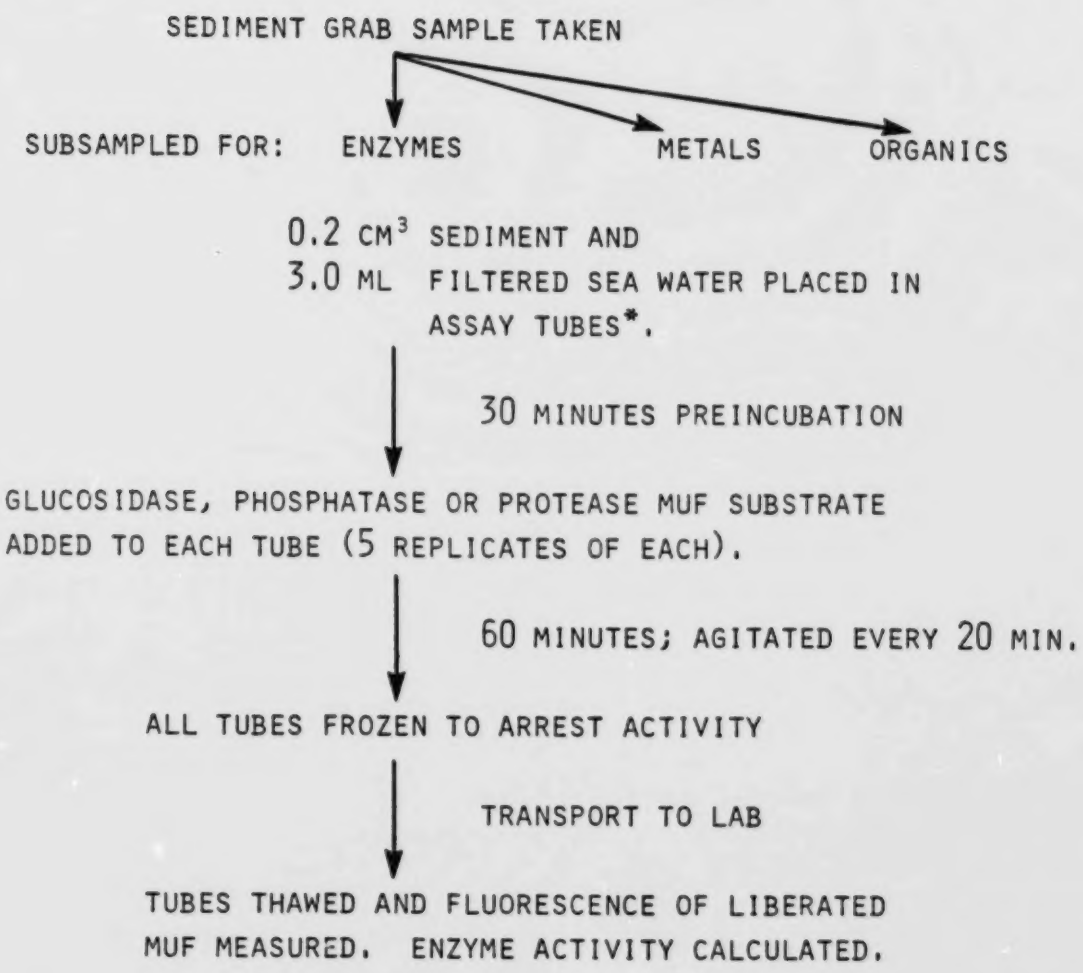

ENZYME ASSAY PROCEDURE
SEDIMENT GRAB SAMPLE TAKEN
SUBSAMPLED FOR:
ENZYMES
METALS
ORGANICS
0.2 CM3 SEDIMENT AND
3.0 ML FILTERED SEA WATER PLACED IN
ASSAY TUBES*.
30 MINUTES PREINCUBATION
GLUCOSIDASE, PHOSPHATASE OR PROTEASE MUF SUBSTRATE
ADDED TO EACH TUBE (5 REPLICATES OF EACH).
60 MINUTES; AGITATED EVERY 20 MIN.
ALL TUBES FROZEN TO ARREST ACTIVITY
TRANSPORT TO LAB
TUBES THAWED AND FLUORESCENCE OF LIBERATED
MUF MEASURED. ENZYME ACTIVITY CALCULATED.
* 1.0 UM MUF STANDARD ADDED TO DUPLICATE TUBES TO PROVIDE
INTERNAL STANDARDS.

Figure 3: Flow chart of sediment exoenzyme activity assay.

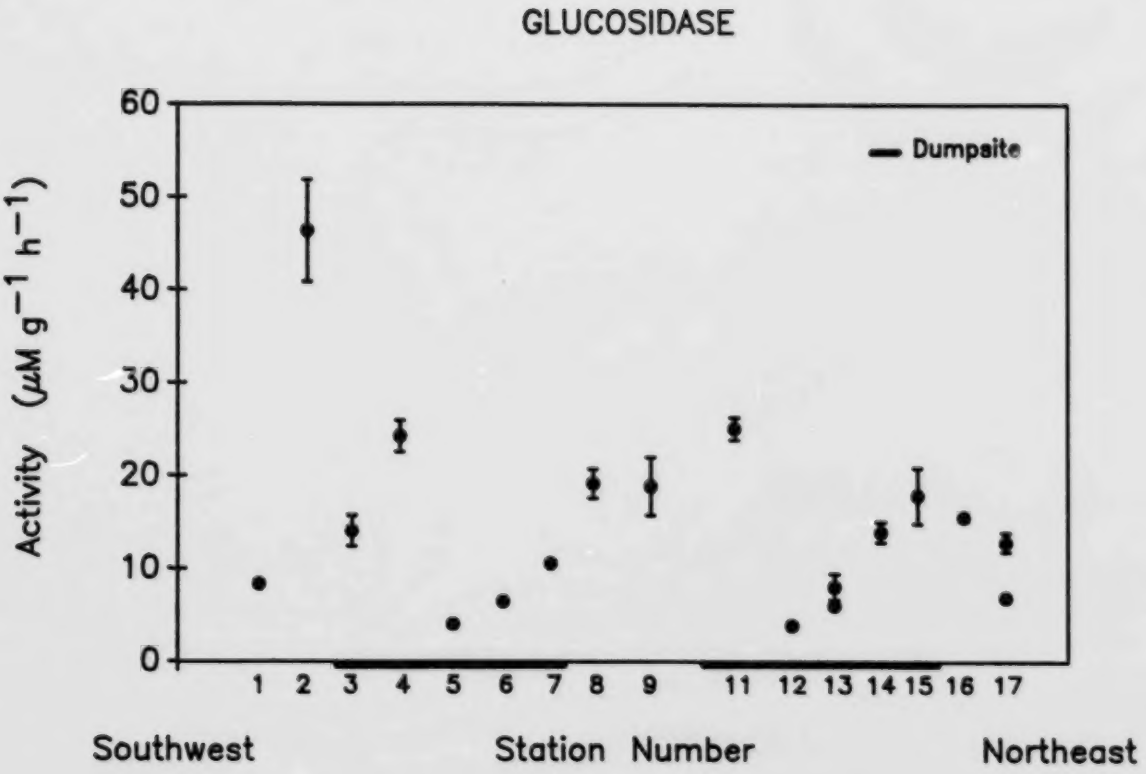


Figure 4: Glucosidase activity (mean + /- S.E.) along an east- west transect through both sediment dumpsites in Pictou Harbour, NS.

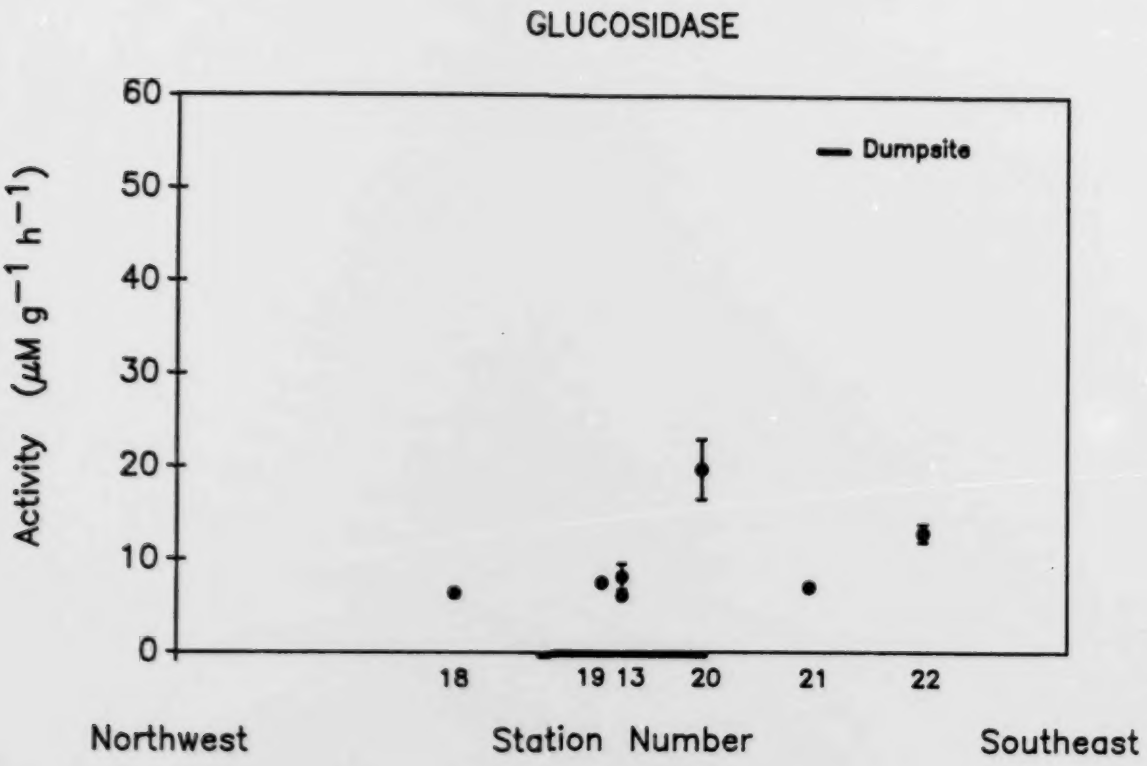


Figure 5: Glucosidase activity (mean + /-S.E.) along a north-south transect across the east dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 4.

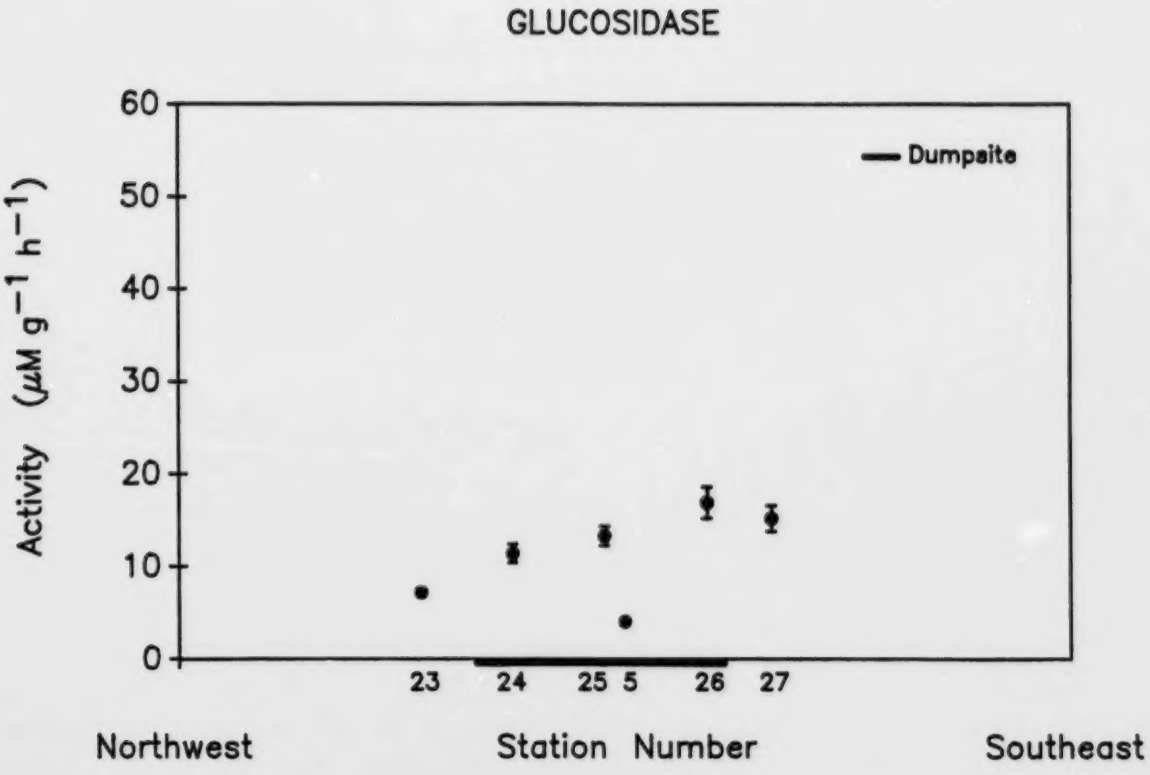


Figure 6: Glucosidase activity (mean + /- S.E.) along a north- south transect across the west dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 4.

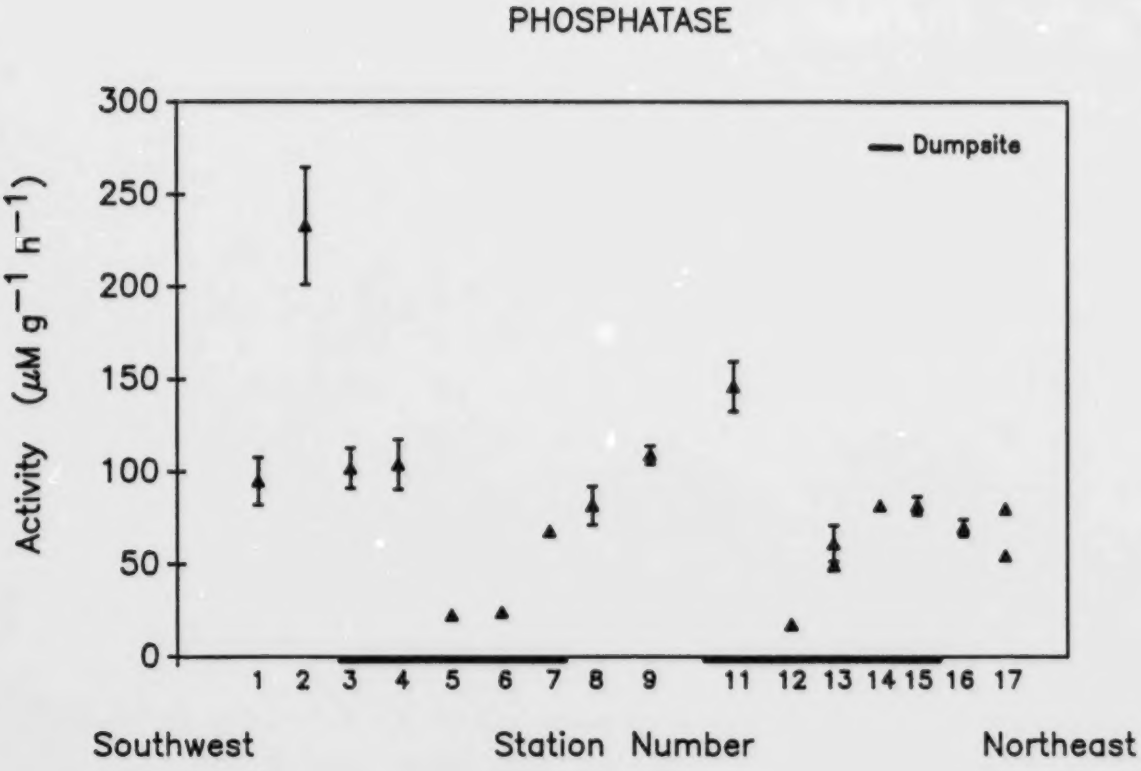


Figure 7: Phosphatase activity (mean + /- S.E.) along an east- west transect through both sediment dumpsites in Pictou Harbour, NS.

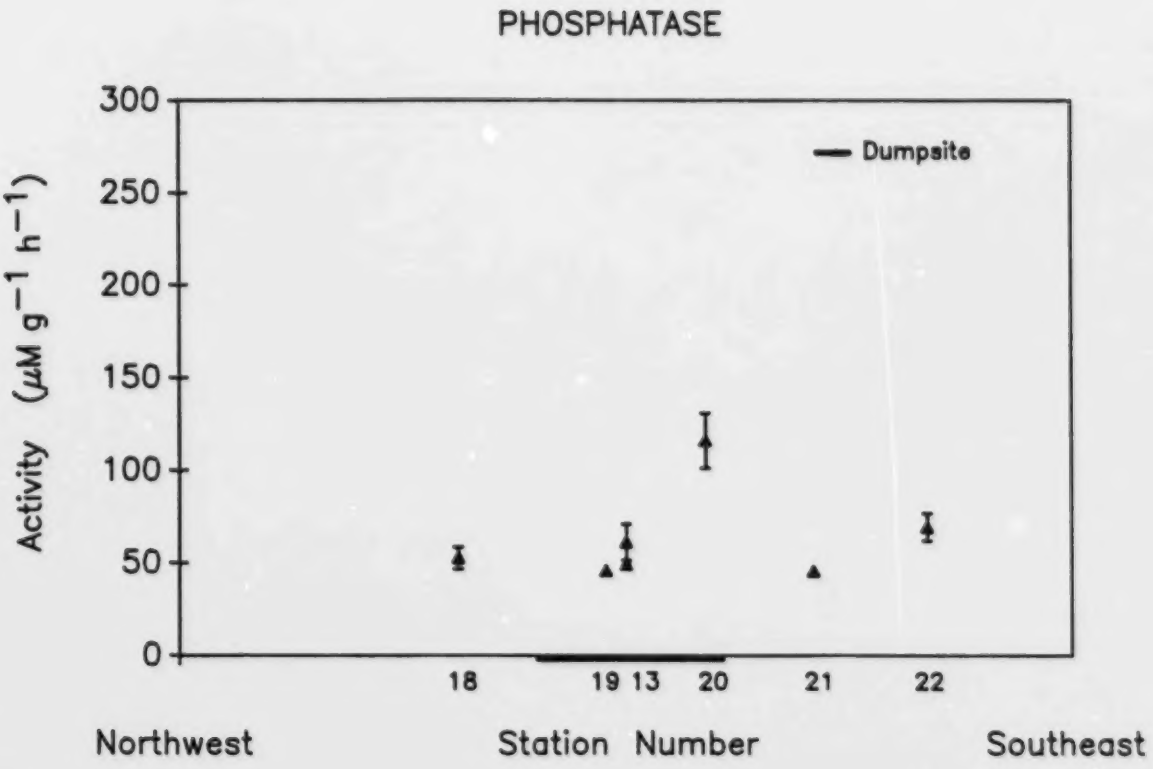


Figure 8: Phosphatase activity (mean + /- S.E.) along a north- south transect across the east dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 7.

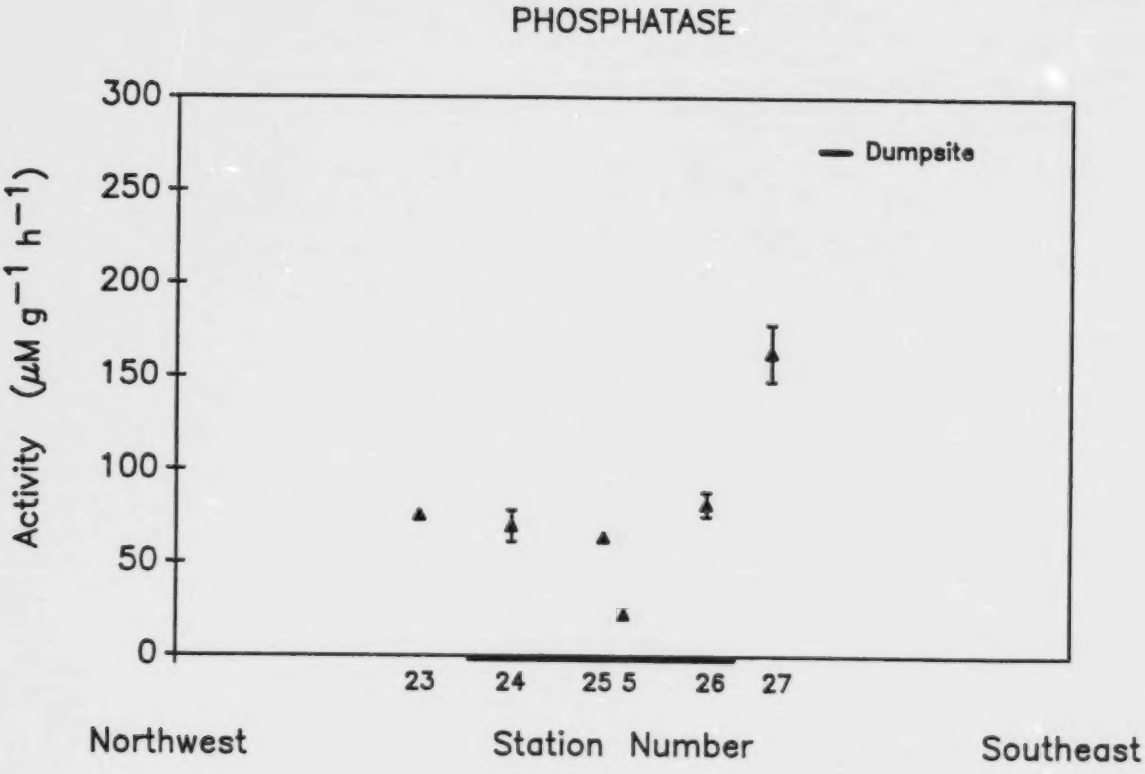


Figure 9: Phosphatase activity (mean +/- S.E.) along a north- south transect across the west dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 7.

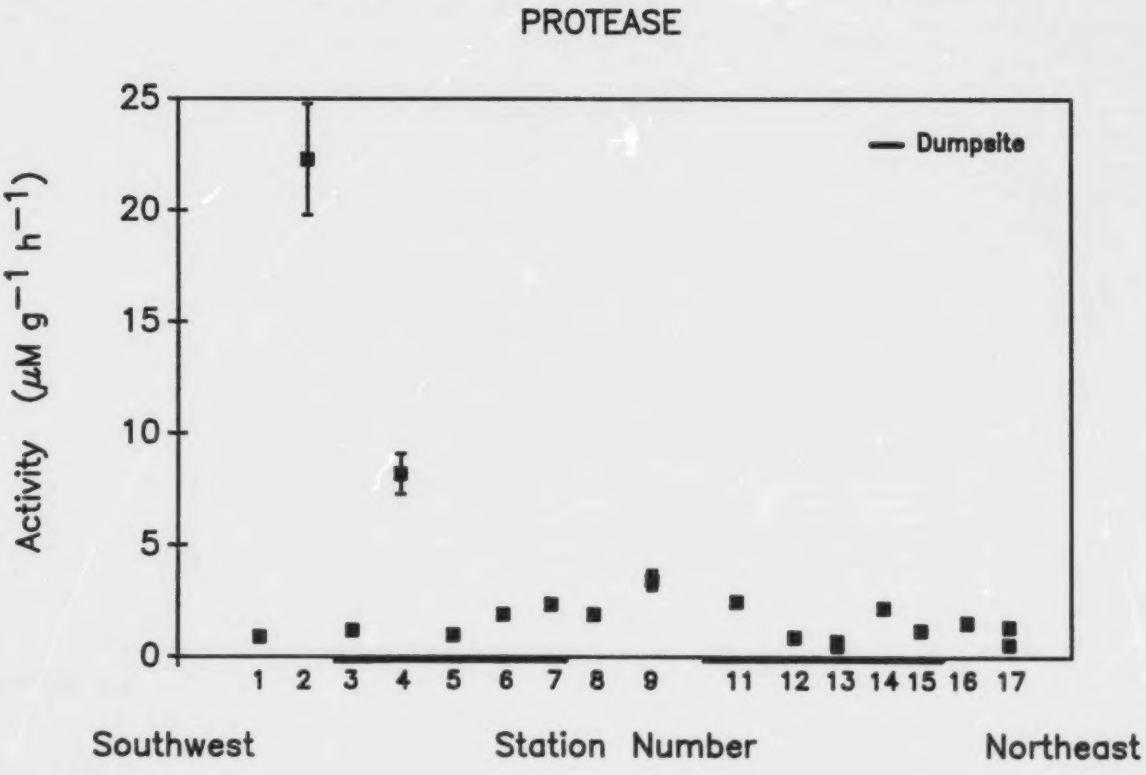


Figure 10: Protease activity (mean +/- S.E.) along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

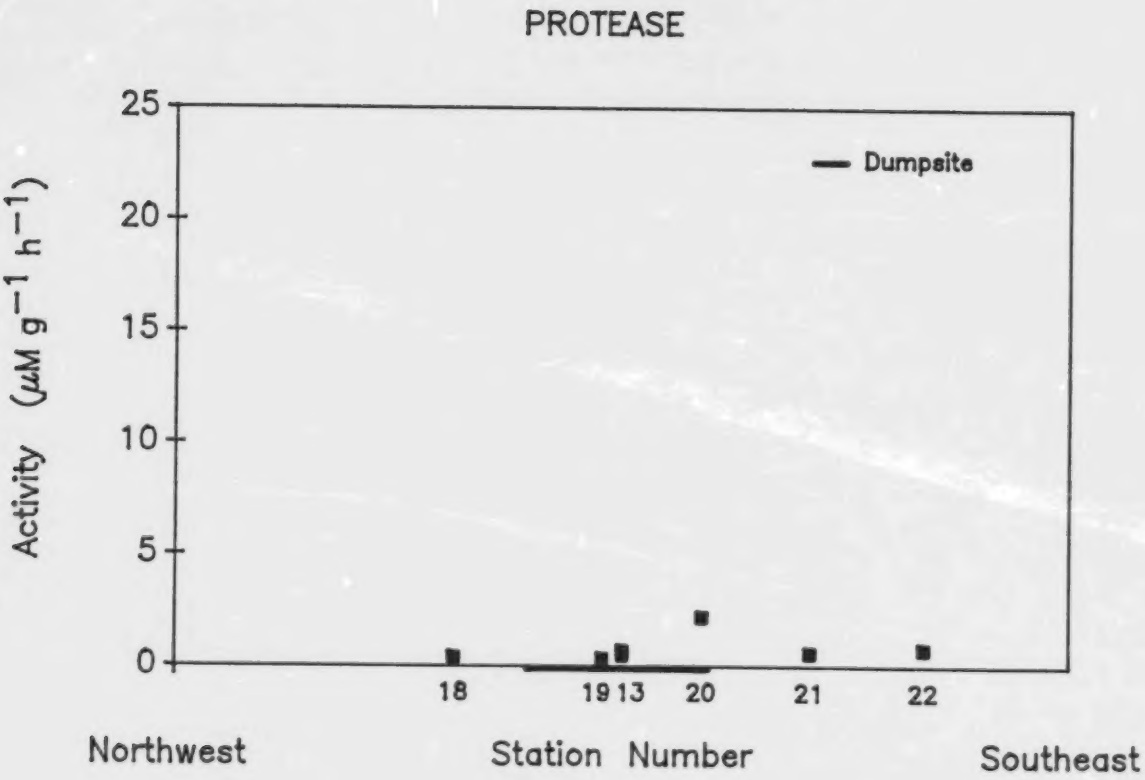


Figure 11: Protease activity (mean + /- S.E.) along a north-south transect across the east dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 10.

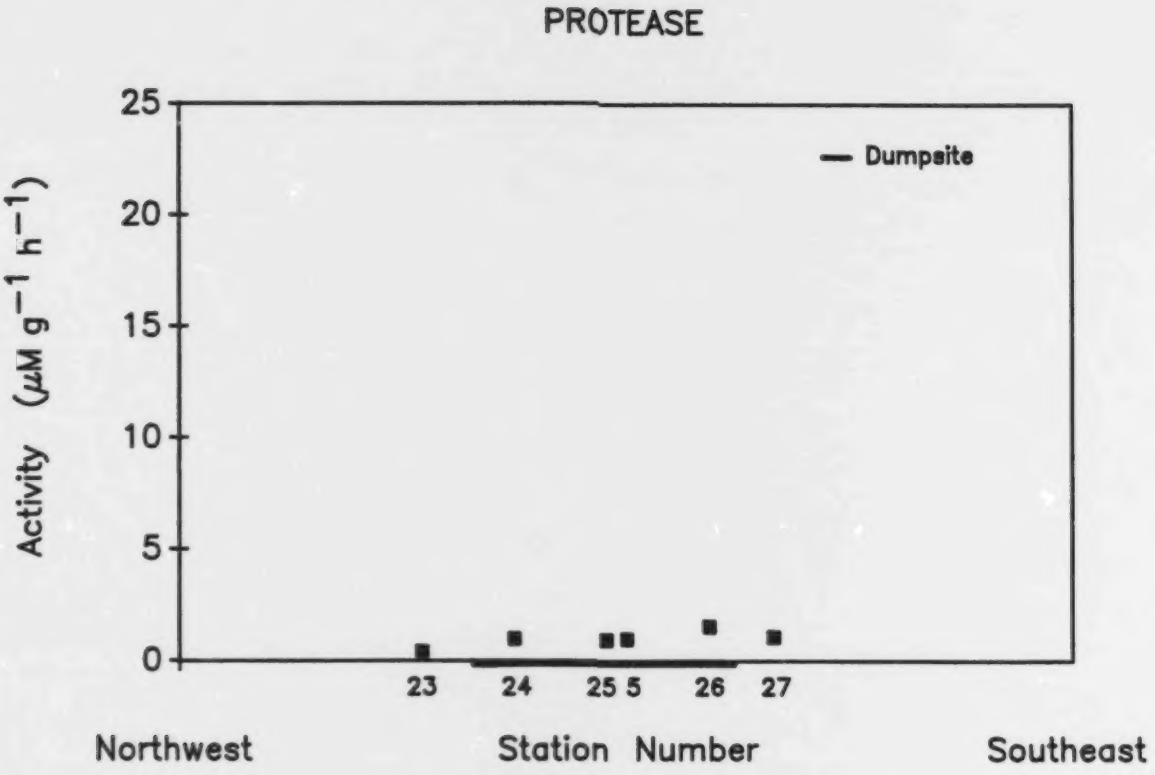


Figure 12: Protease activity (mean + /- S.E.) along a north-south transect across the west dumpsite in Pictou Harbour, NS. Note: X-axis not to same scale as Fig. 10.

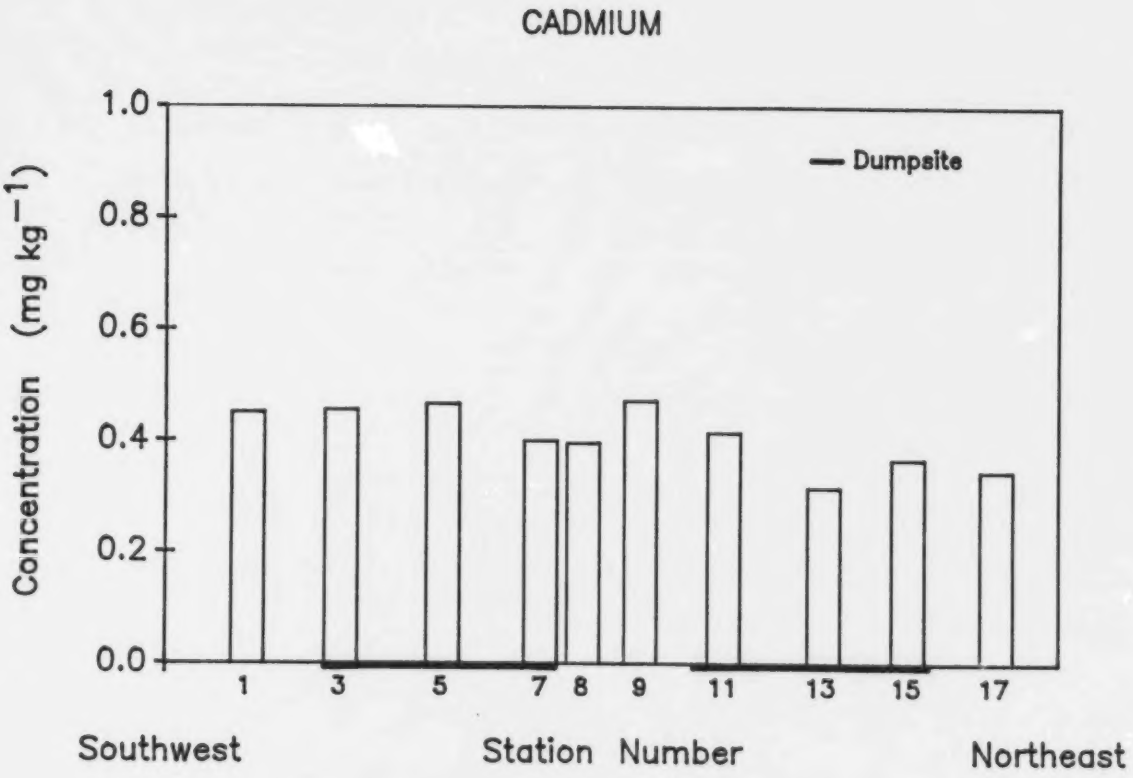


Figure 13: Sediment total cadmium concentrations along an east- west transect through both sediment dumpsites in Pictou Harbour, NS.

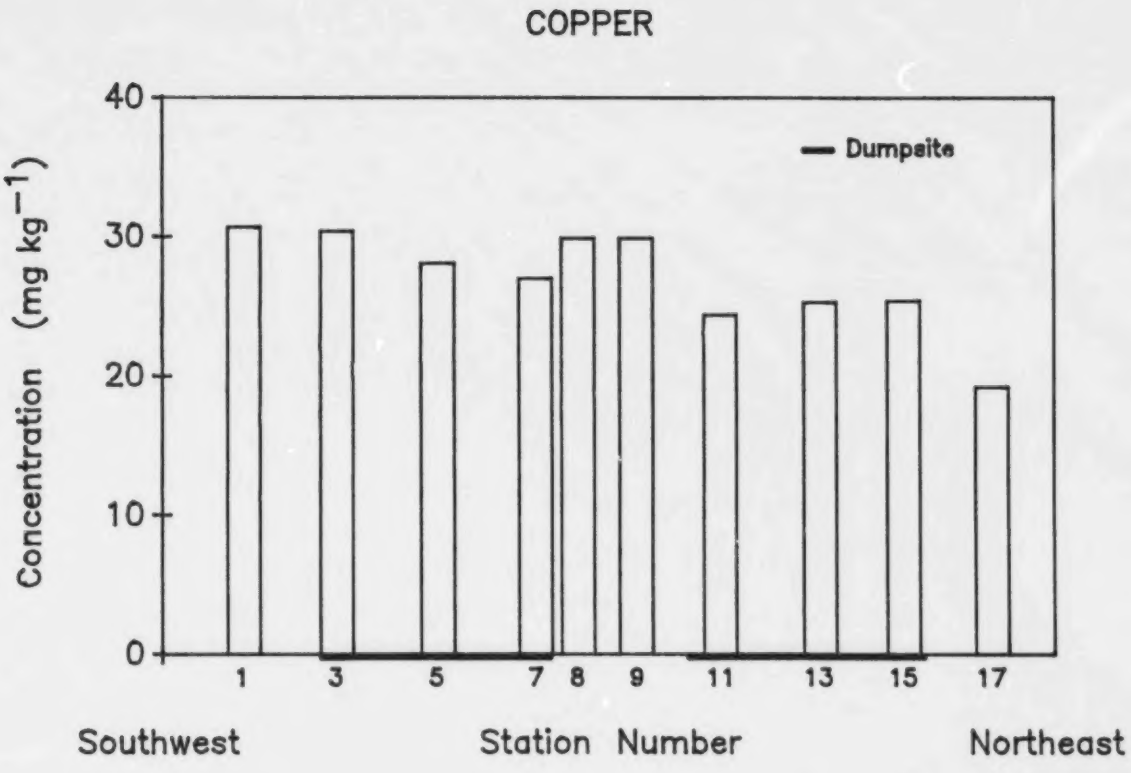


Figure 14: Sediment total copper concentrations along an east- west transect through both sediment dumpsites in Pictou Harbour, NS.

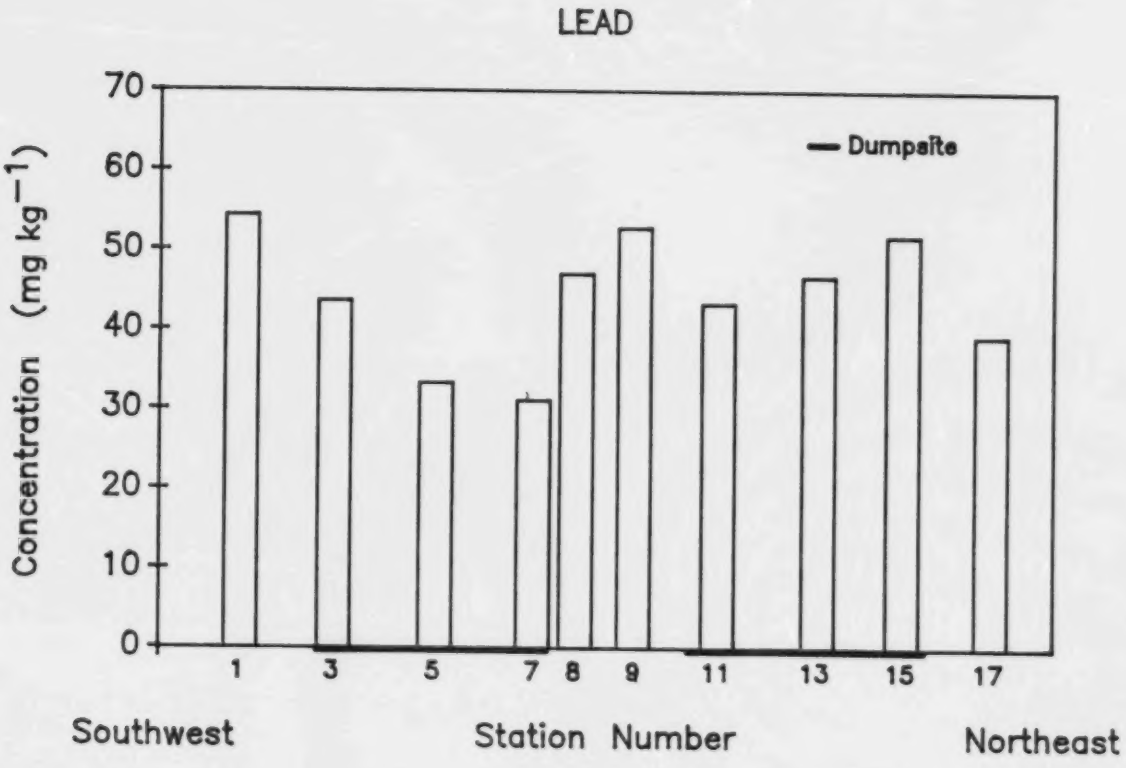


Figure 15: Sediment total lead concentrations along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

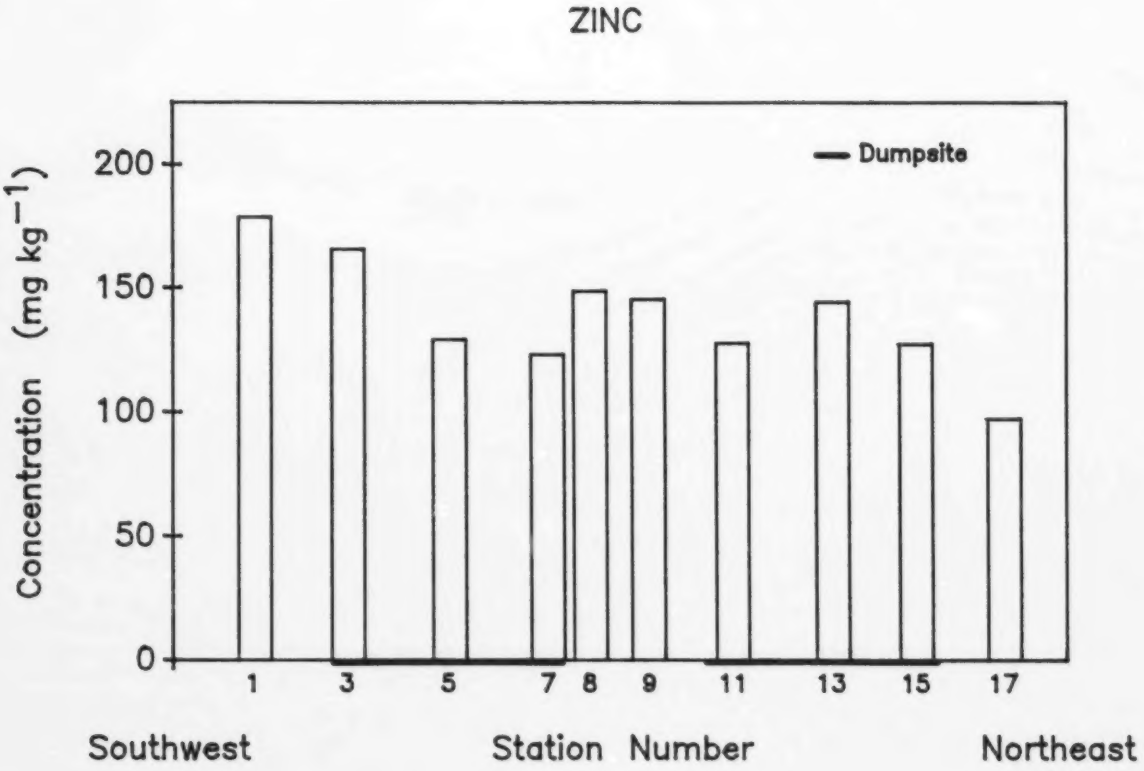


Figure 16: Sediment total zinc concentrations along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

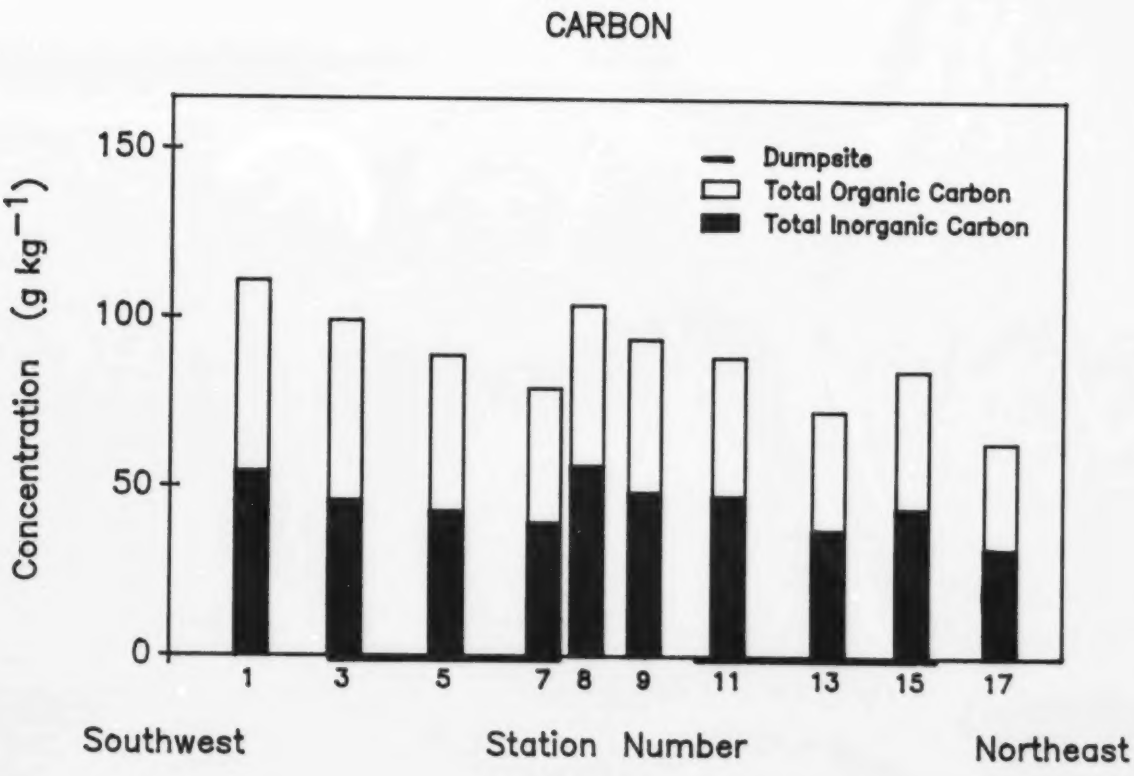


Figure 17: Sediment total inorganic carbon (TIC) and total organic carbon (TOC) concentrations along an east- west transect through both sediment dumpsites in Pictou Harbour, NS.

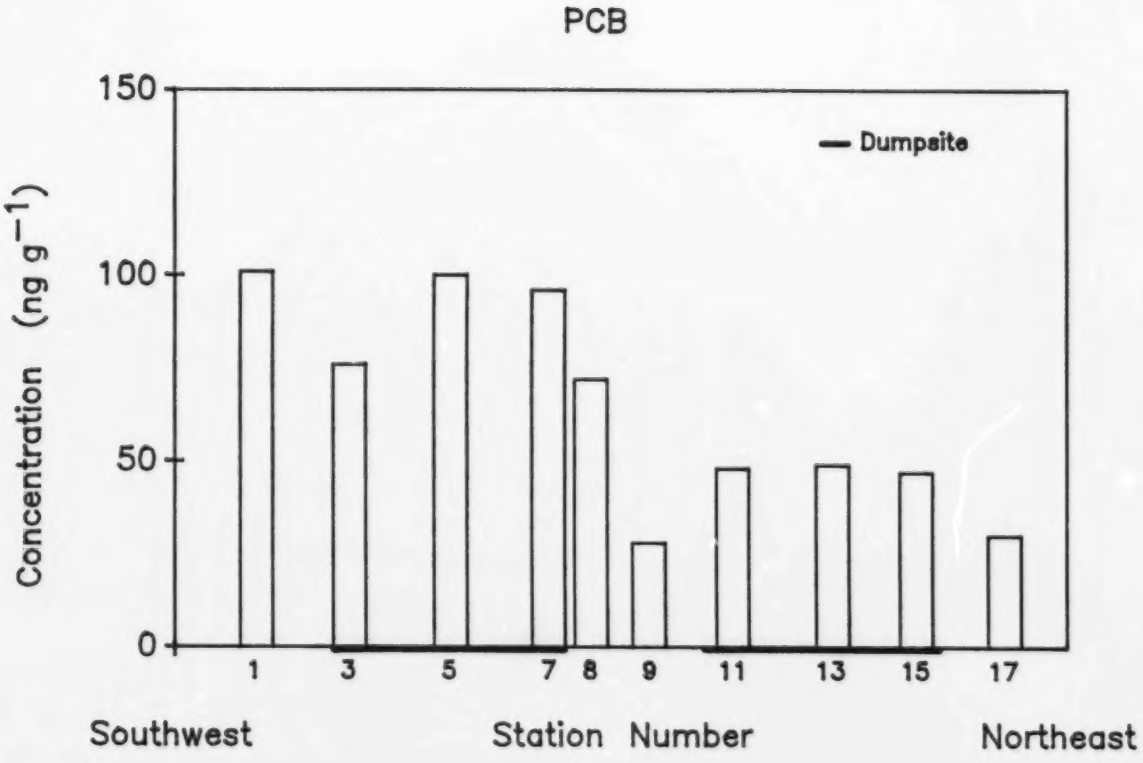


Figure 18: Sediment total PCB concentrations along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

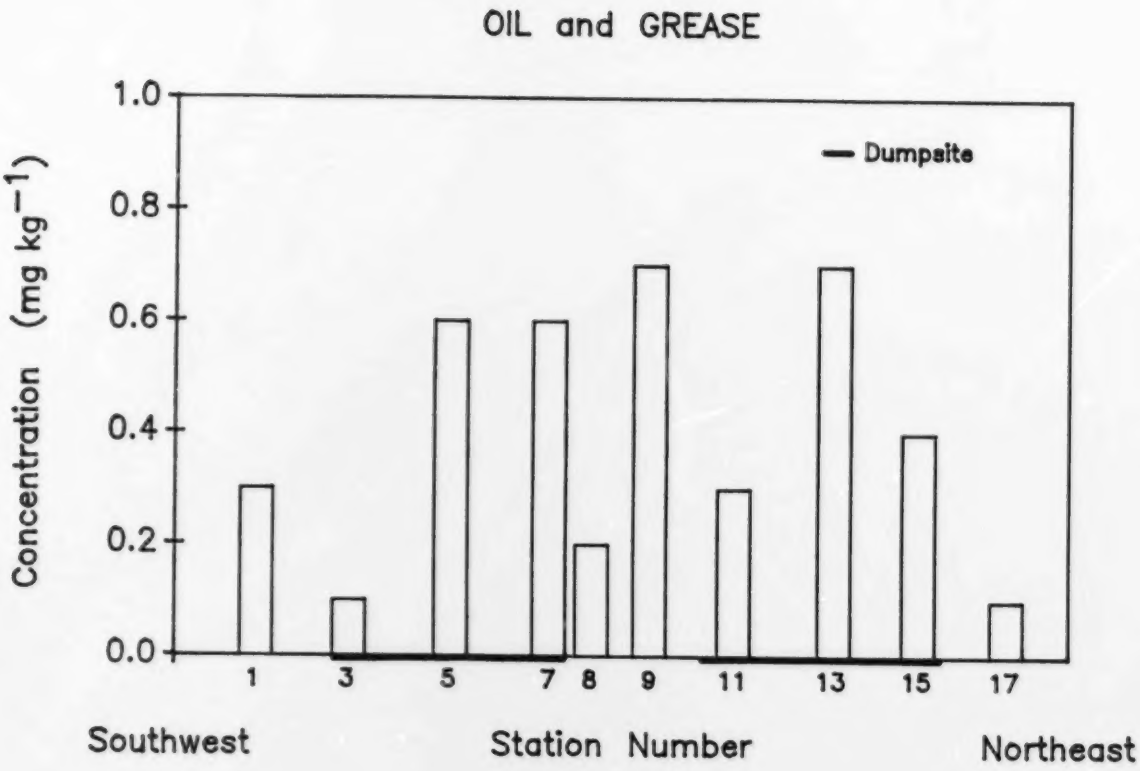


Figure 19: Sediment total oil and grease concentrations along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

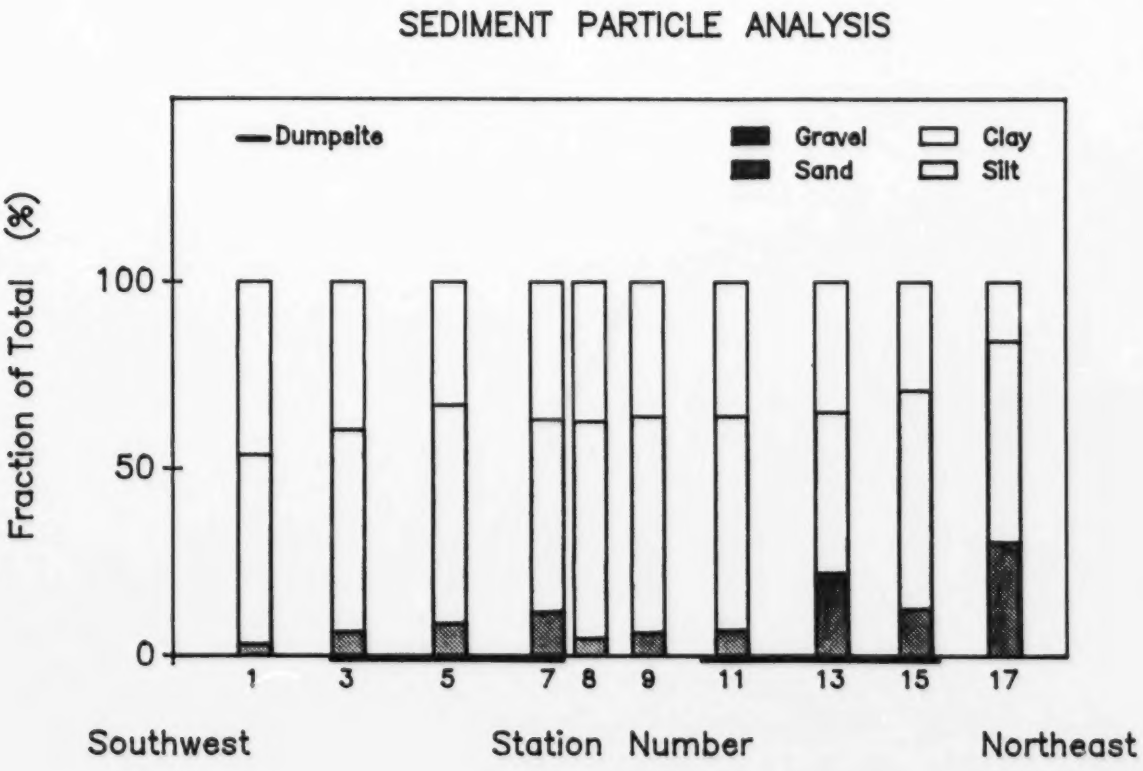


Figure 20: Sediment particle size fractions along an east-west transect through both sediment dumpsites in Pictou Harbour, NS.

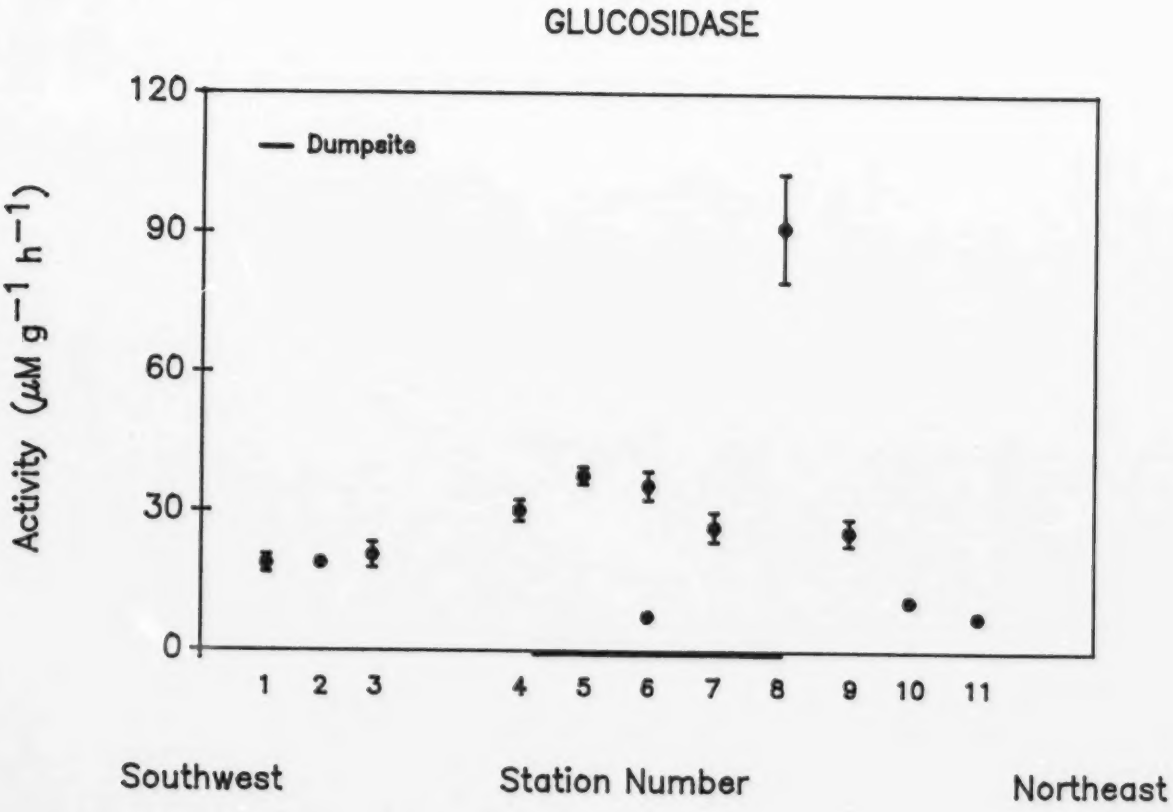


Figure 21: Glucosidase activity (mean +/- S.E.) along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

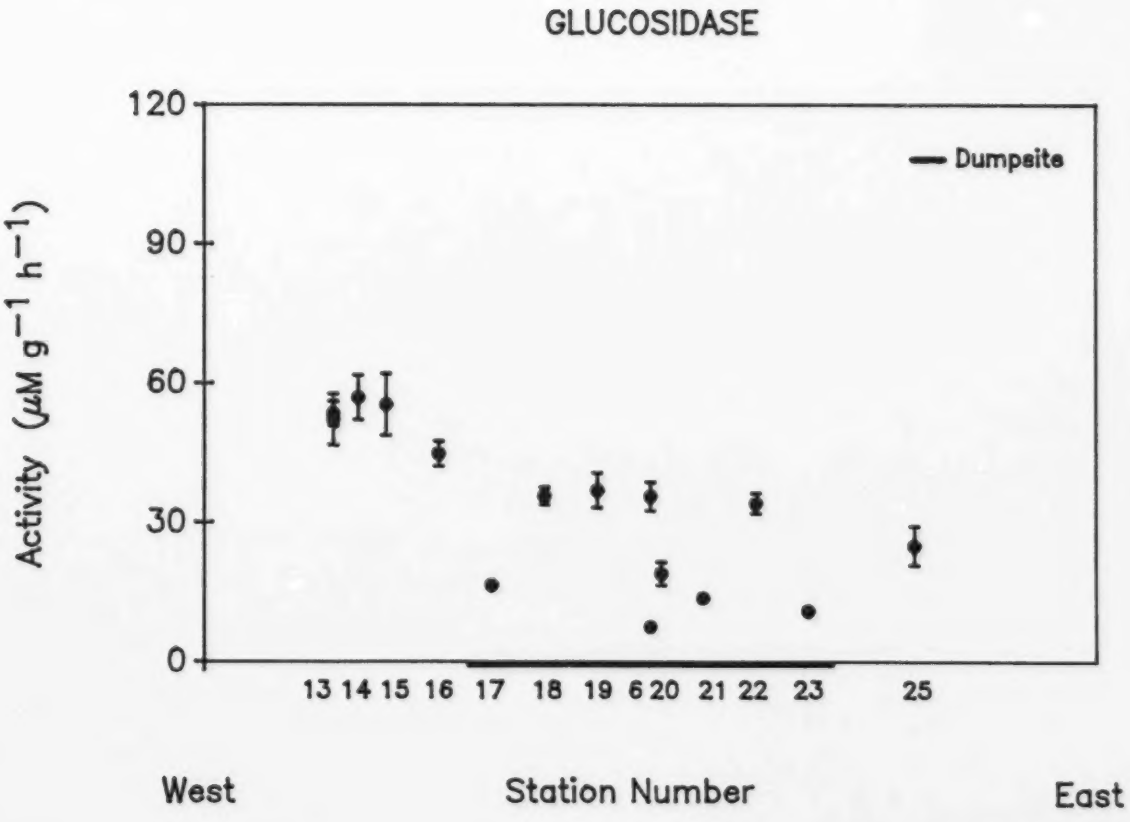


Figure 22: Glucosidase activity (mean +/- S.E.) along a east- west transect through sediment dumpsite "A" in the Miramichi River, NB.

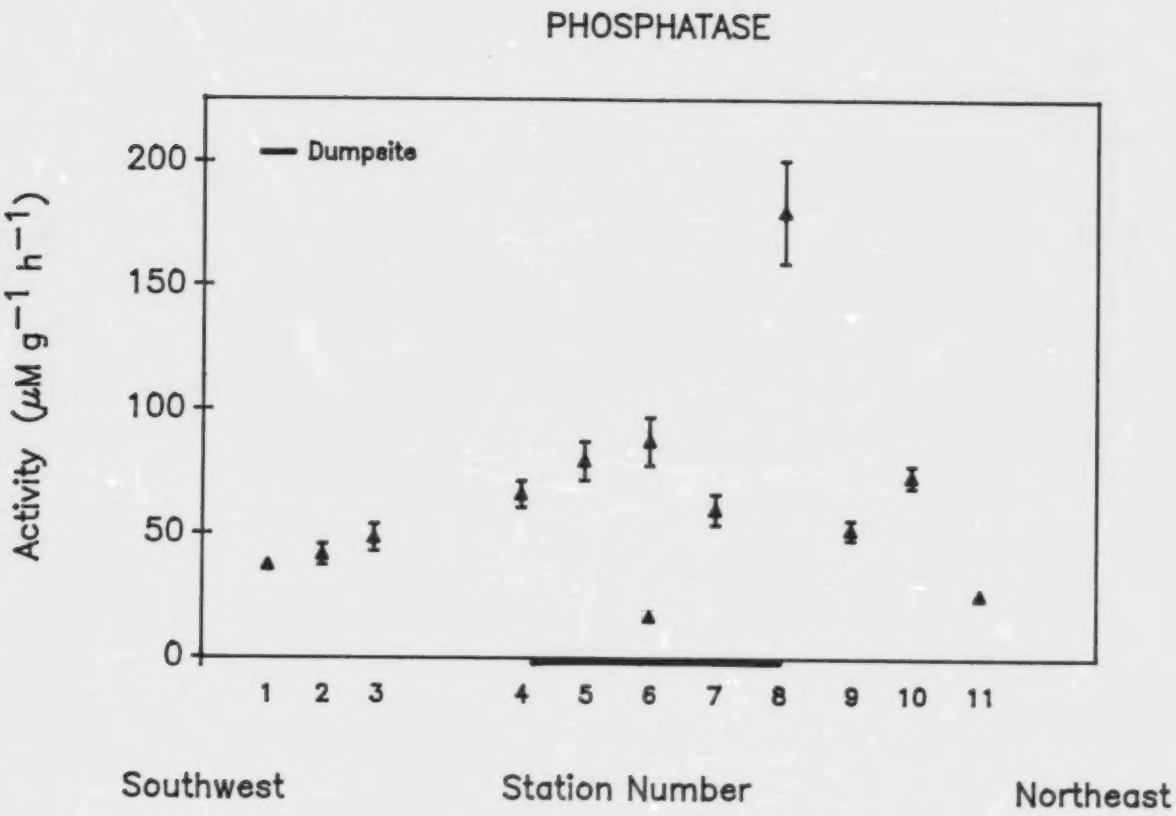


Figure 23: Phosphatase activity (mean +/- S.E.) along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

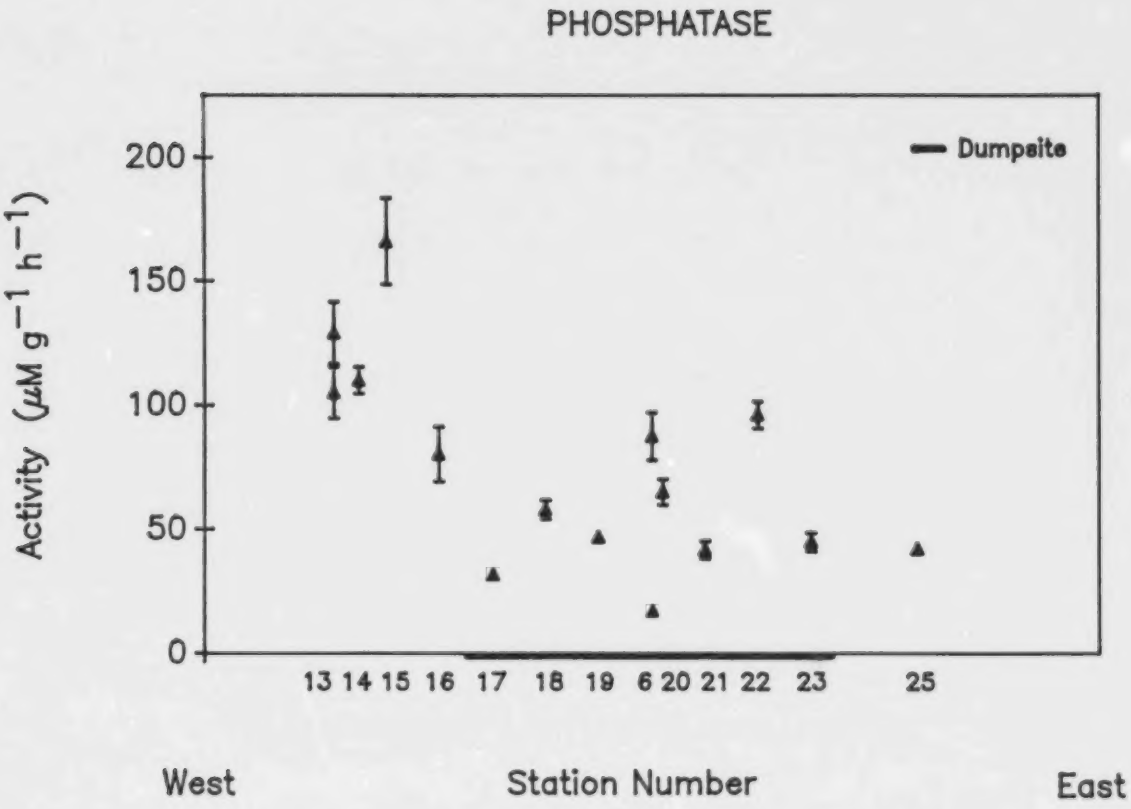


Figure 24: Phosphatase activity (mean +/- S.E.) along a east- west transect through sediment dumpsite "A" in the Miramichi River, NB.

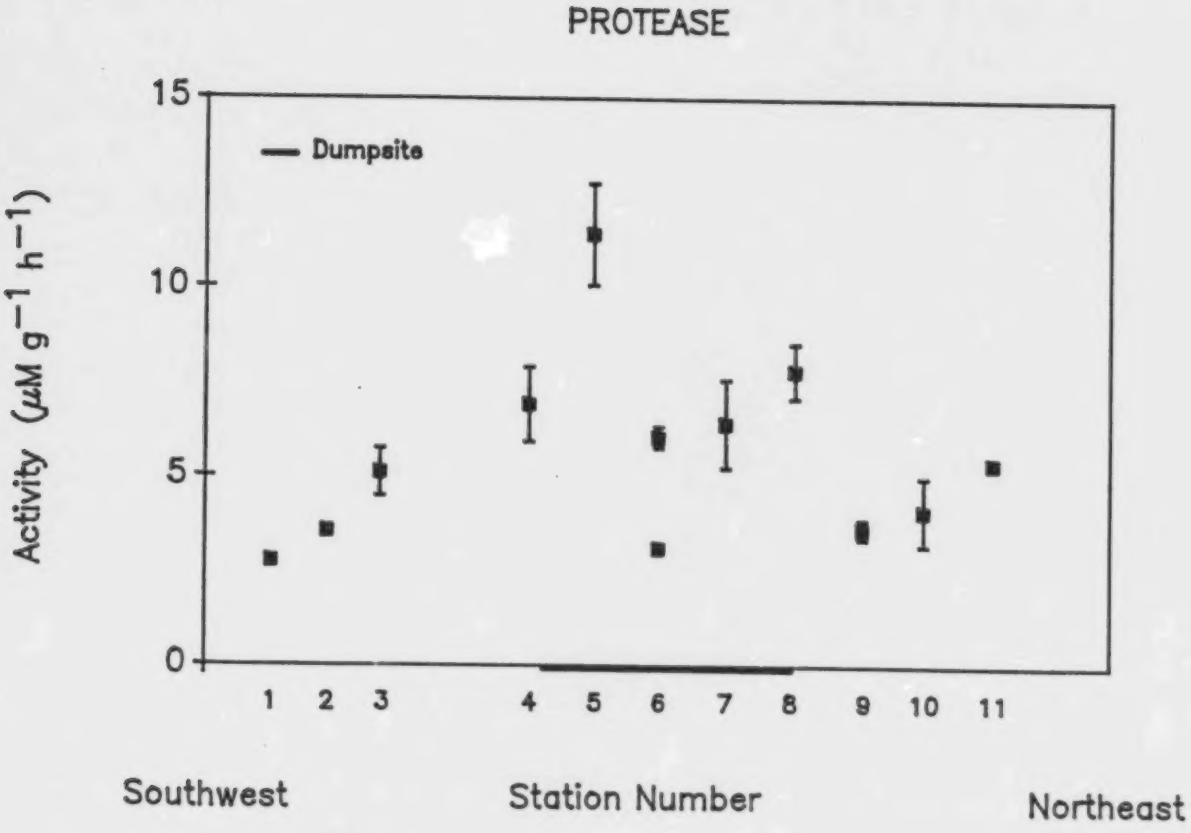


Figure 25: Protease activity (mean + /- S.E.) along a southwest- northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

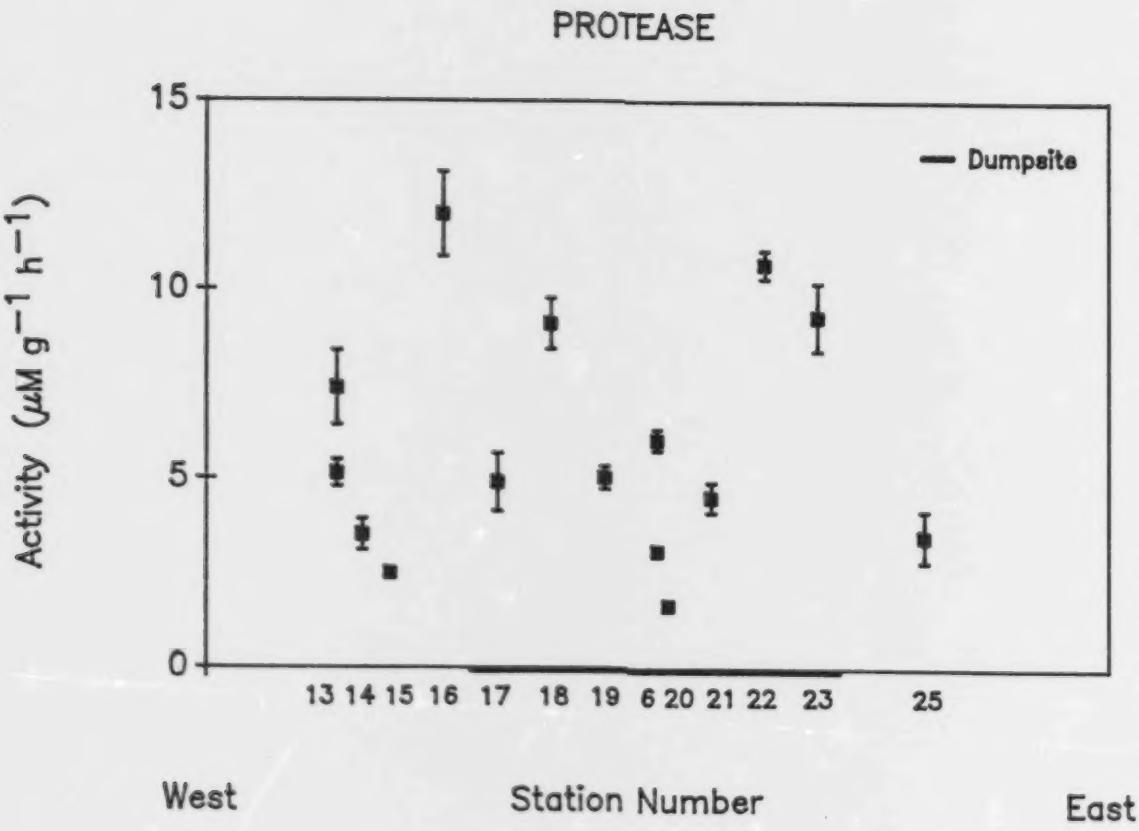


Figure 26: Protease activity (mean + /- S.E.) along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

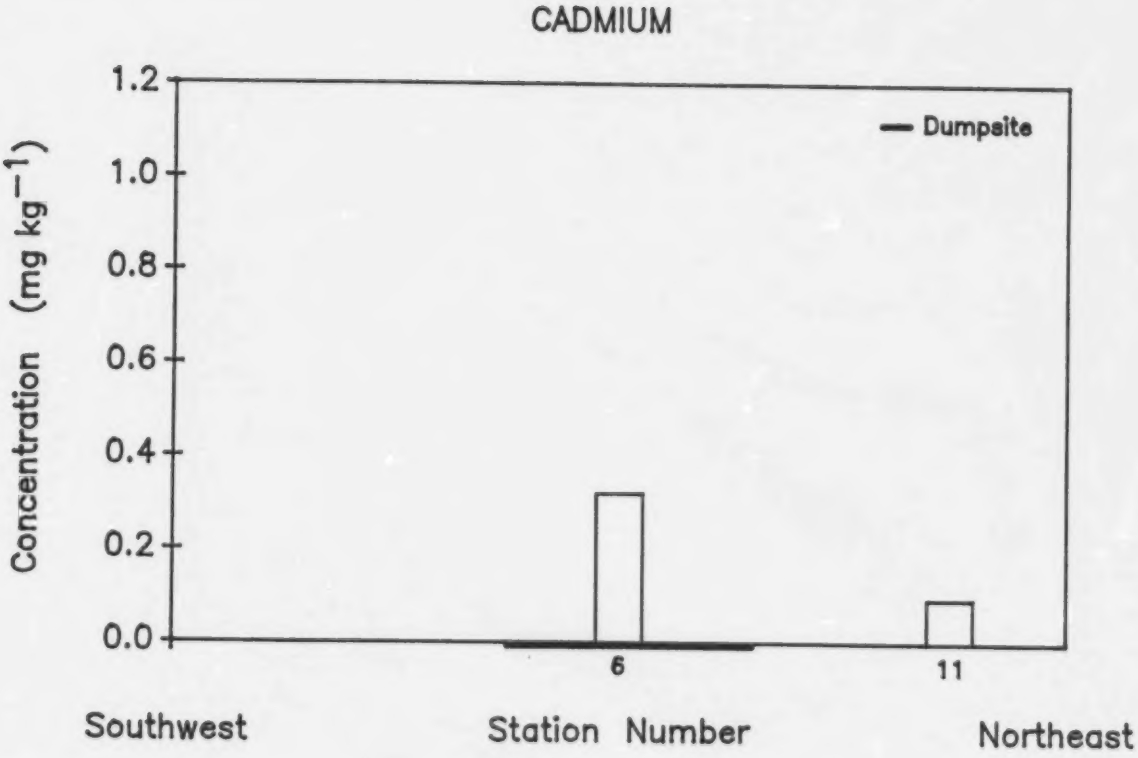


Figure 27: Sediment total cadmium concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

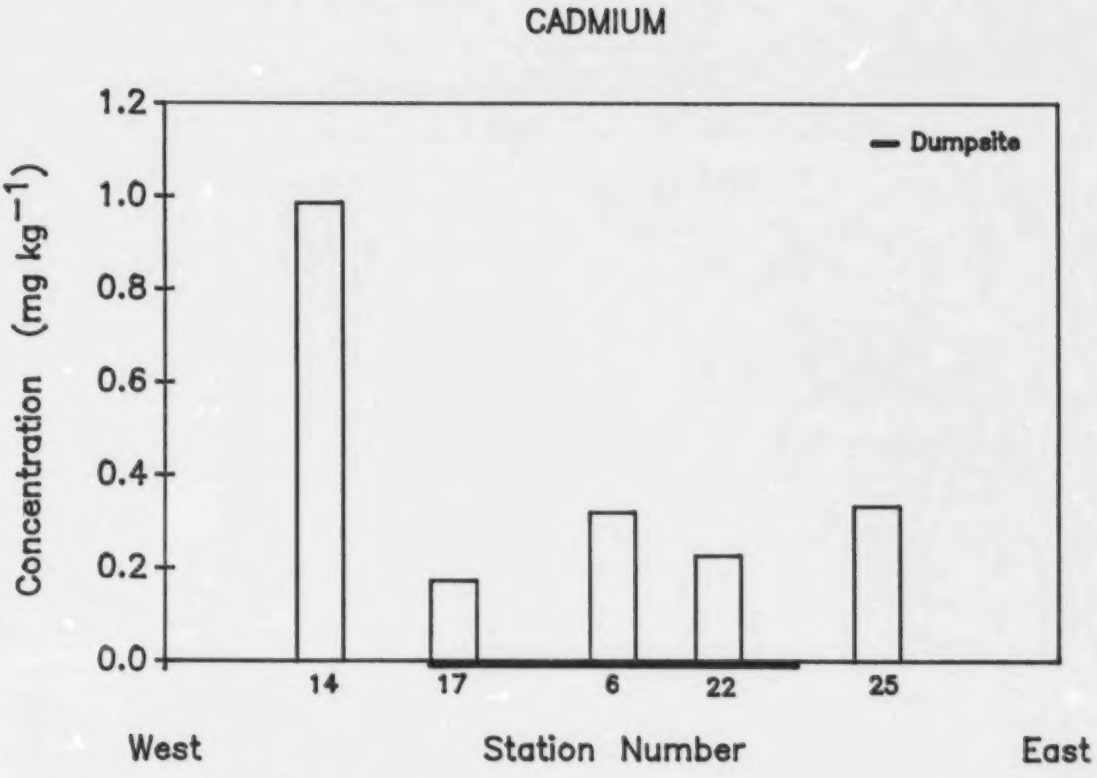


Figure 28: Sediment total cadmium concentrations along a east- west transect through sediment dumpsite "A" in the Miramichi River, NB.

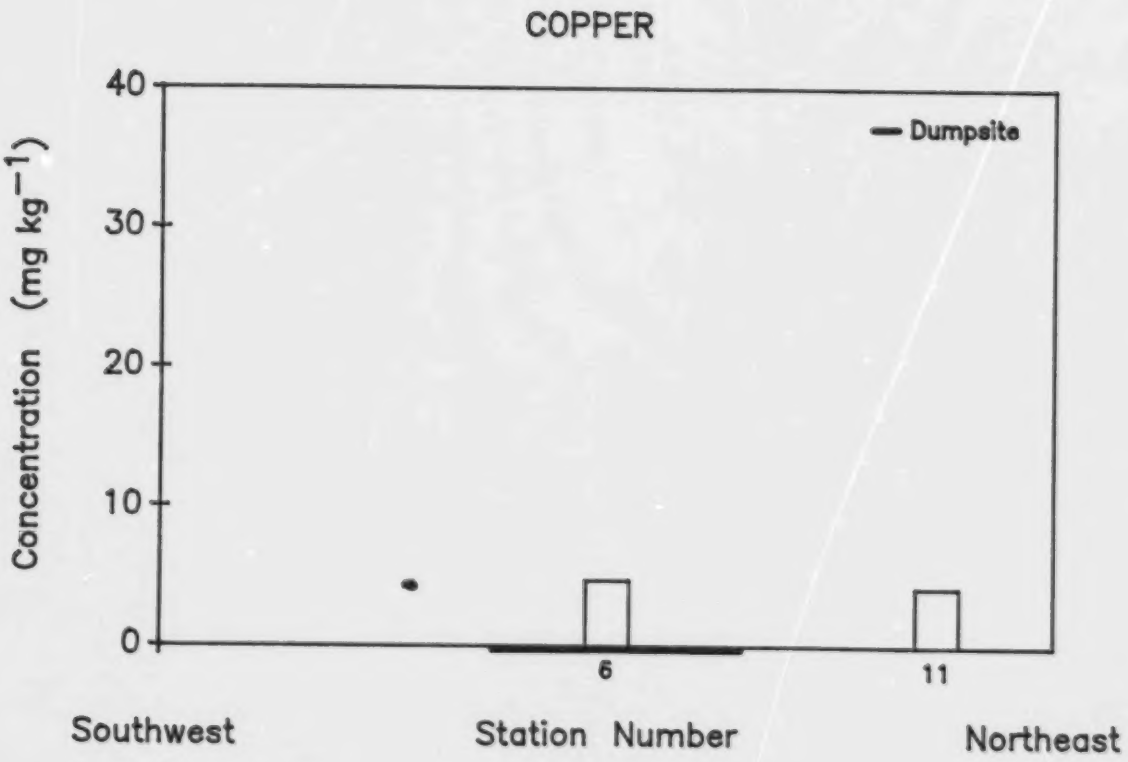


Figure 29: Sediment total copper concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

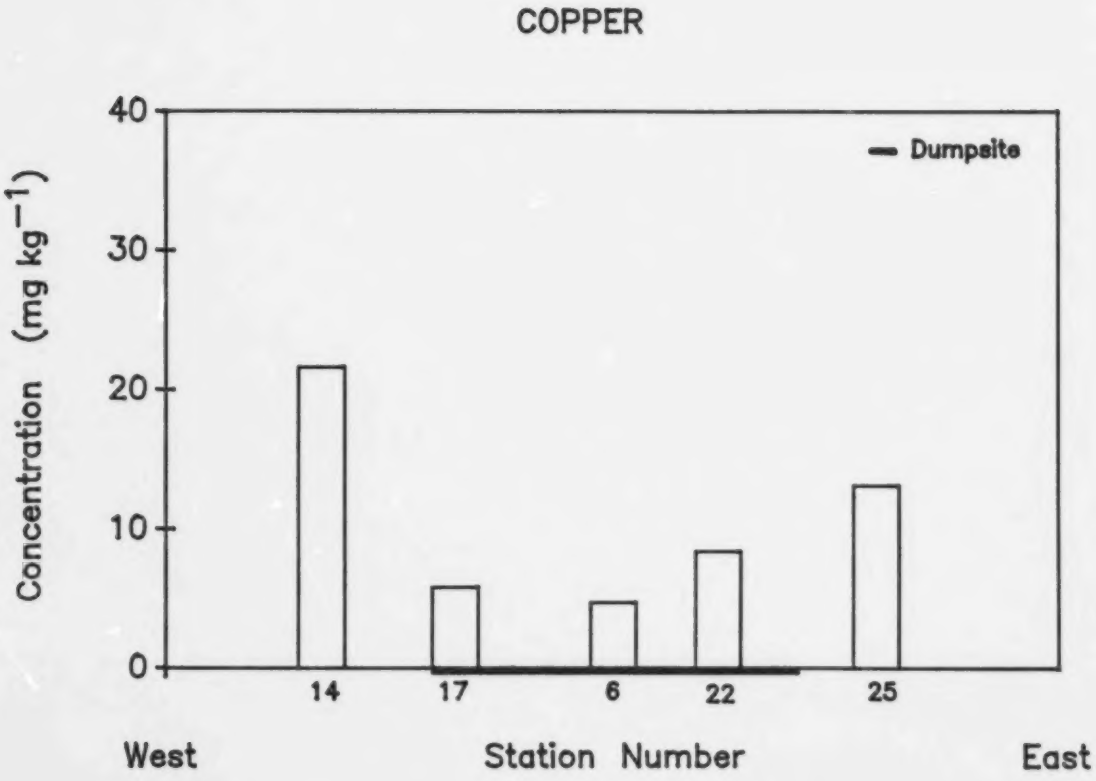


Figure 30: Sediment total copper concentrations along a east- west transect through sediment dumpsite "A" in the Miramichi River, NB.

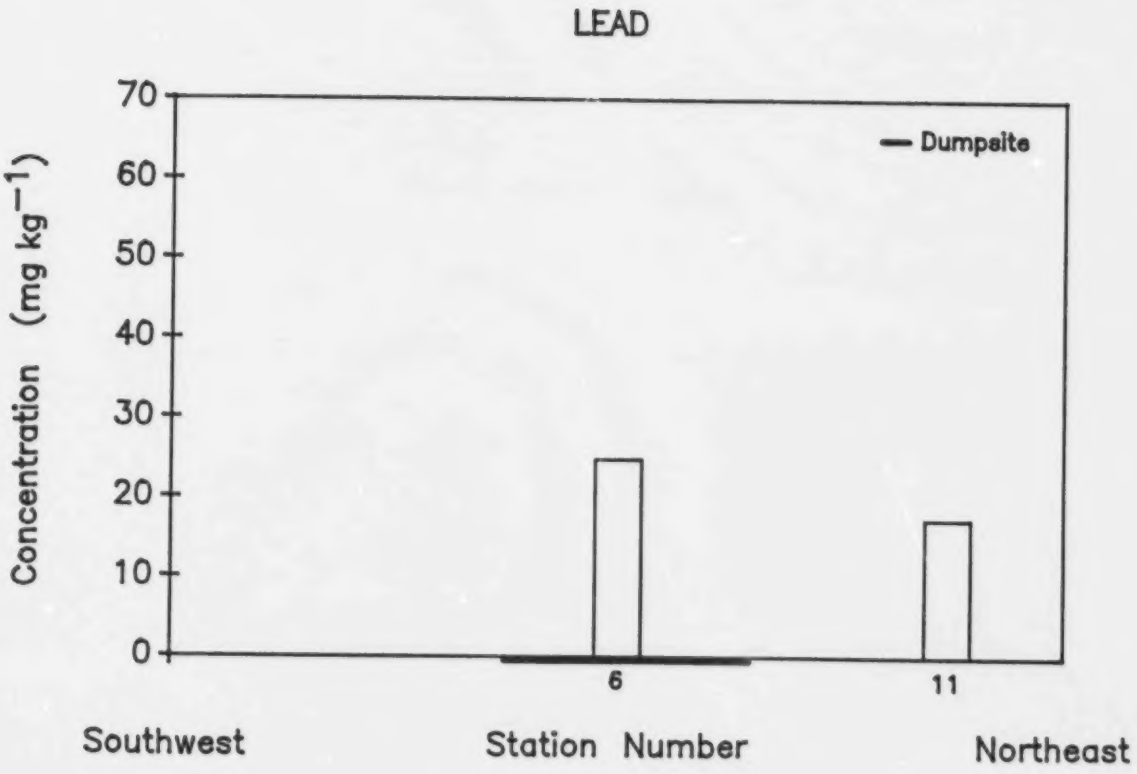


Figure 31: Sediment total lead concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

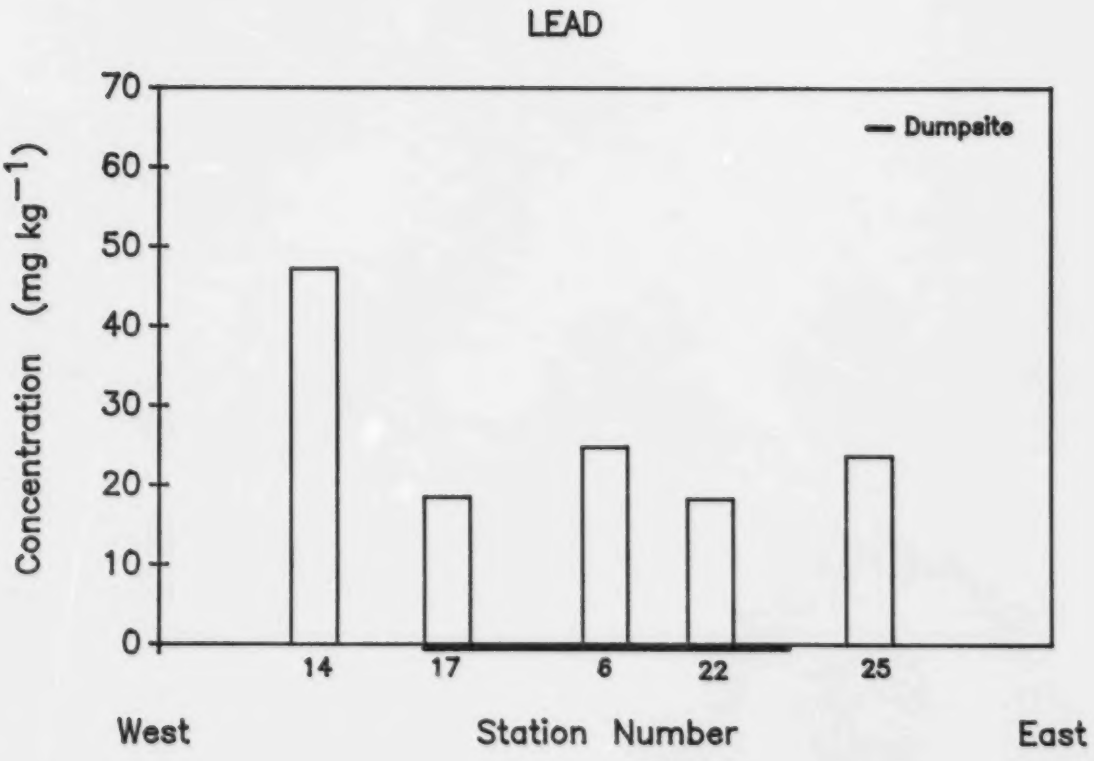


Figure 32: Sediment total lead concentrations along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

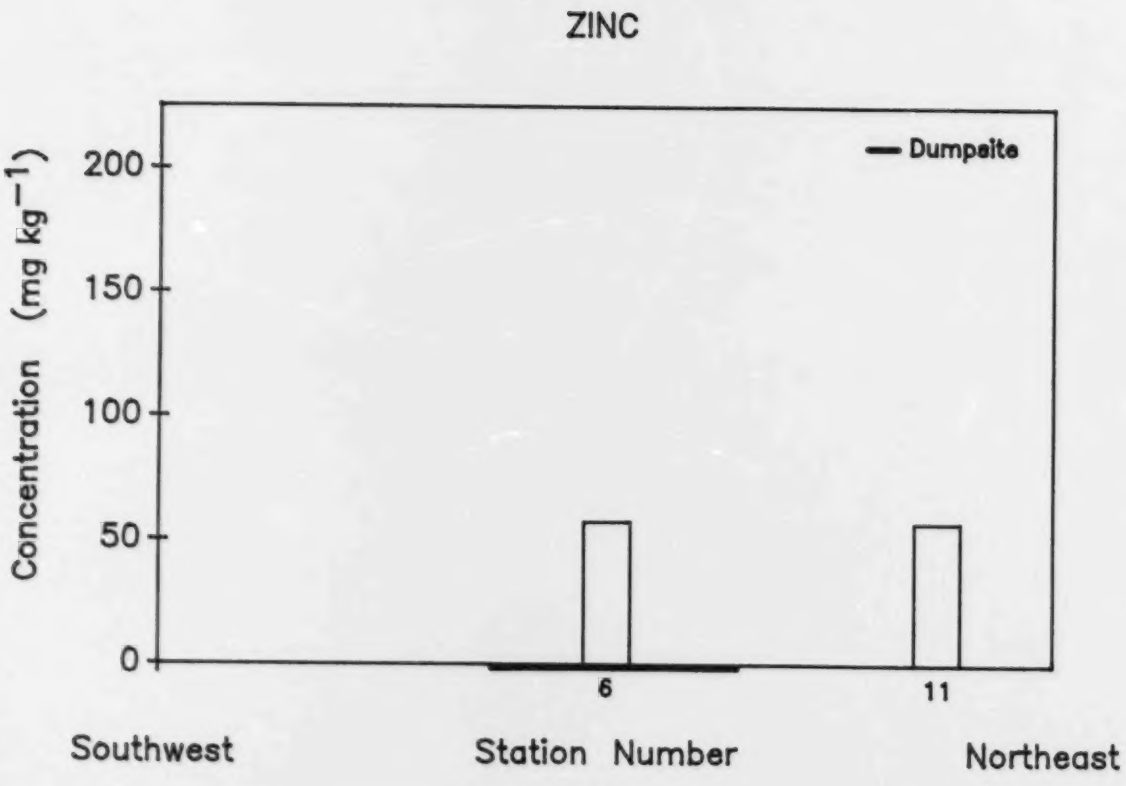


Figure 33: Sediment total zinc concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

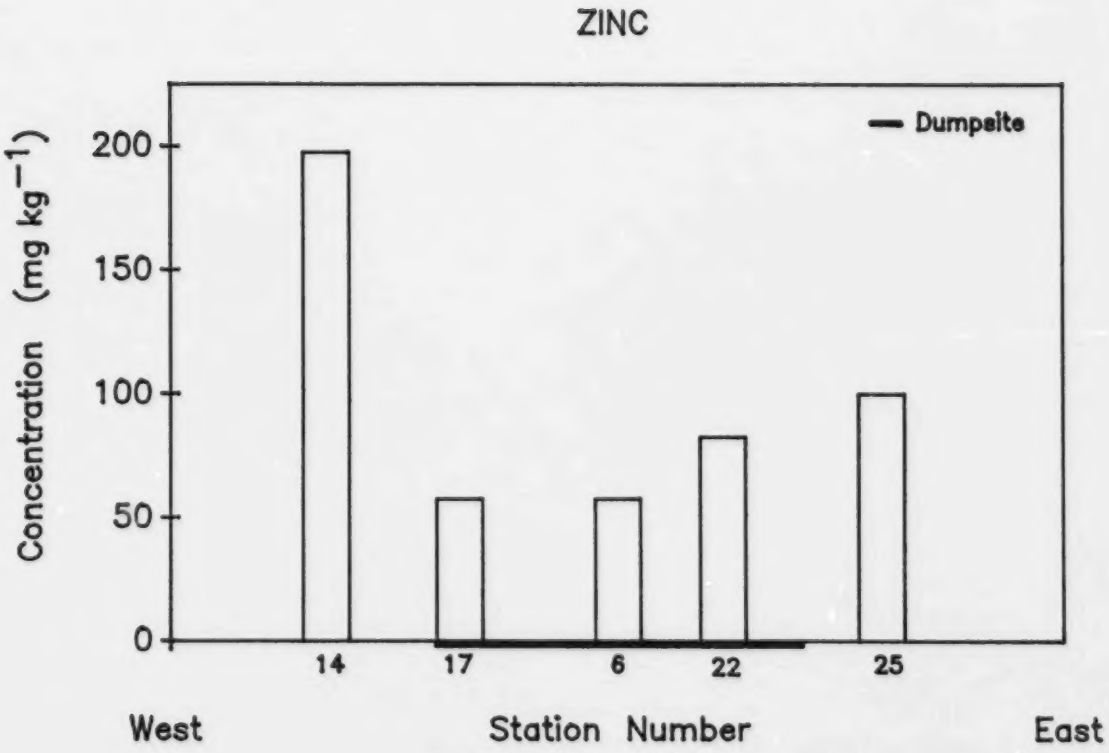


Figure 34: Sediment total zinc concentrations along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

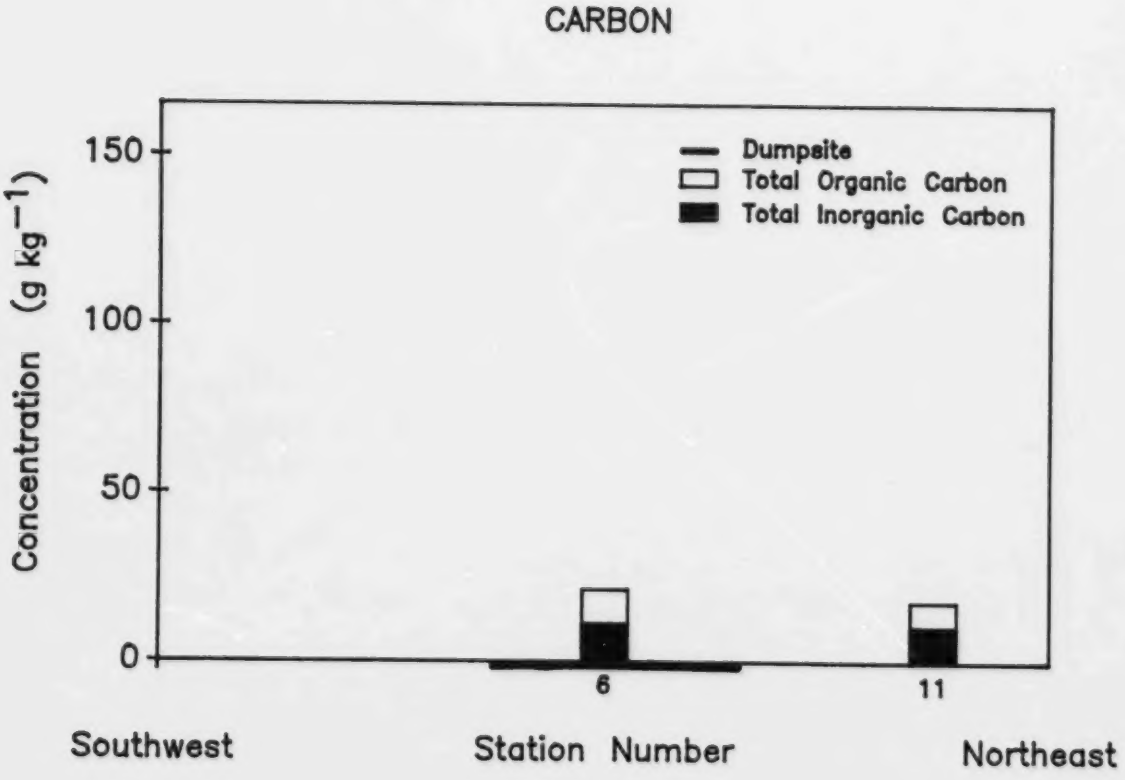


Figure 35: Sediment total inorganic carbon (TIC) and total organic carbon (TOC) concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

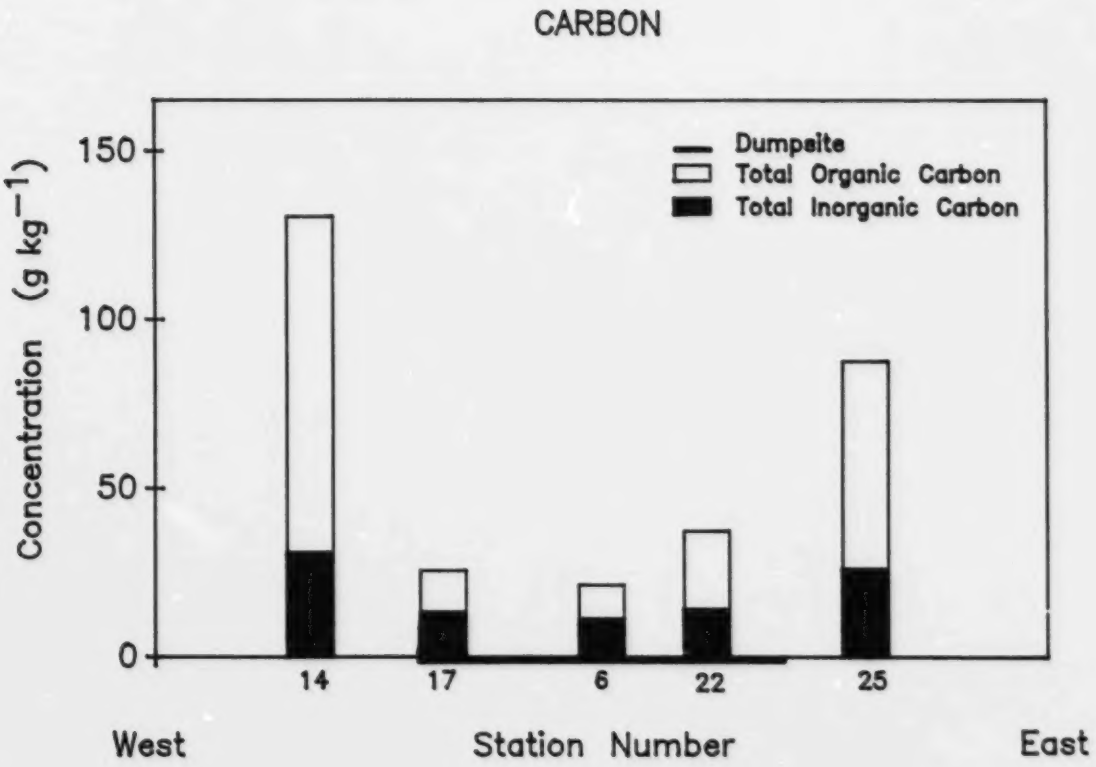


Figure 36: Sediment total inorganic carbon (TIC) and total organic carbon (TOC) concentrations along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

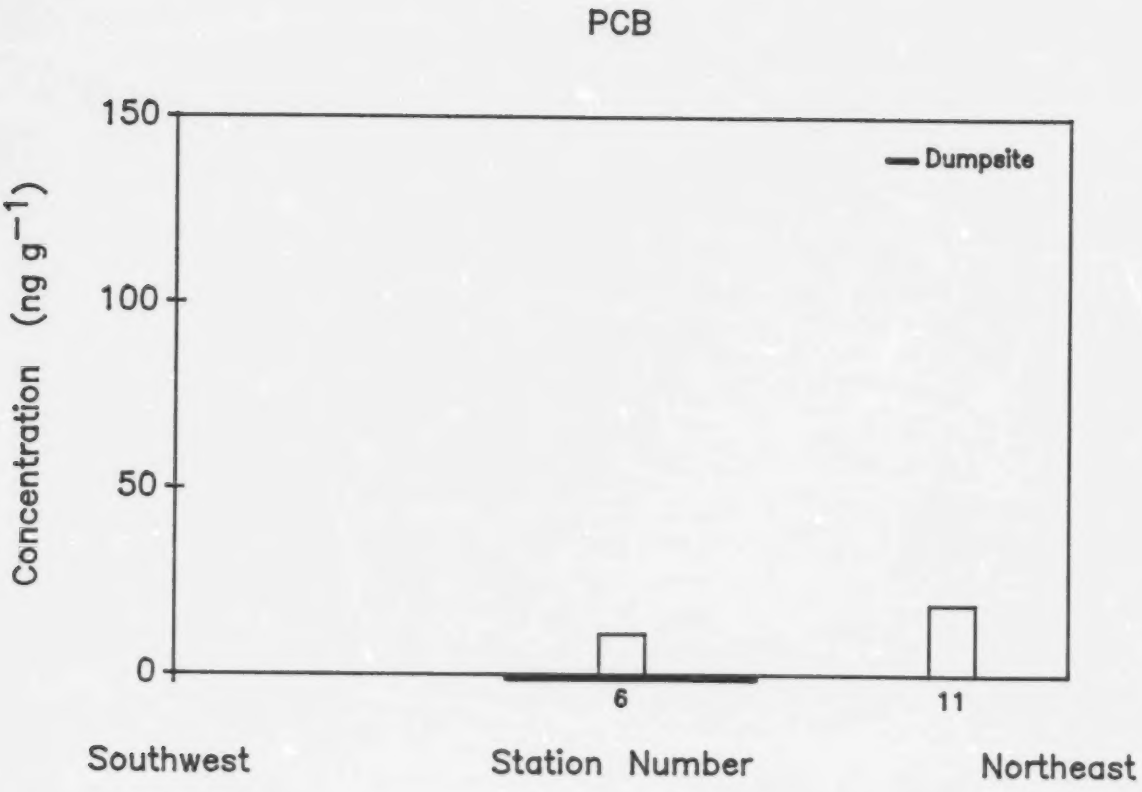


Figure 37: Sediment total PCB concentrations along a southwest- northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

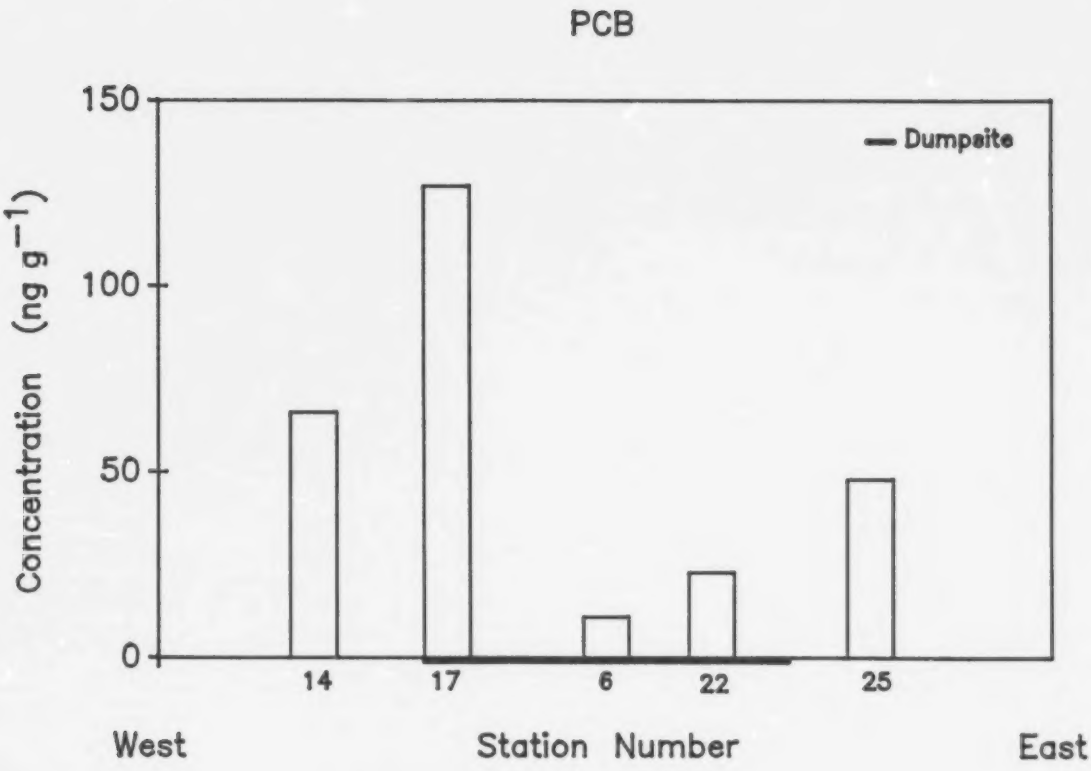


Figure 38: Sediment total PCB concentrations along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

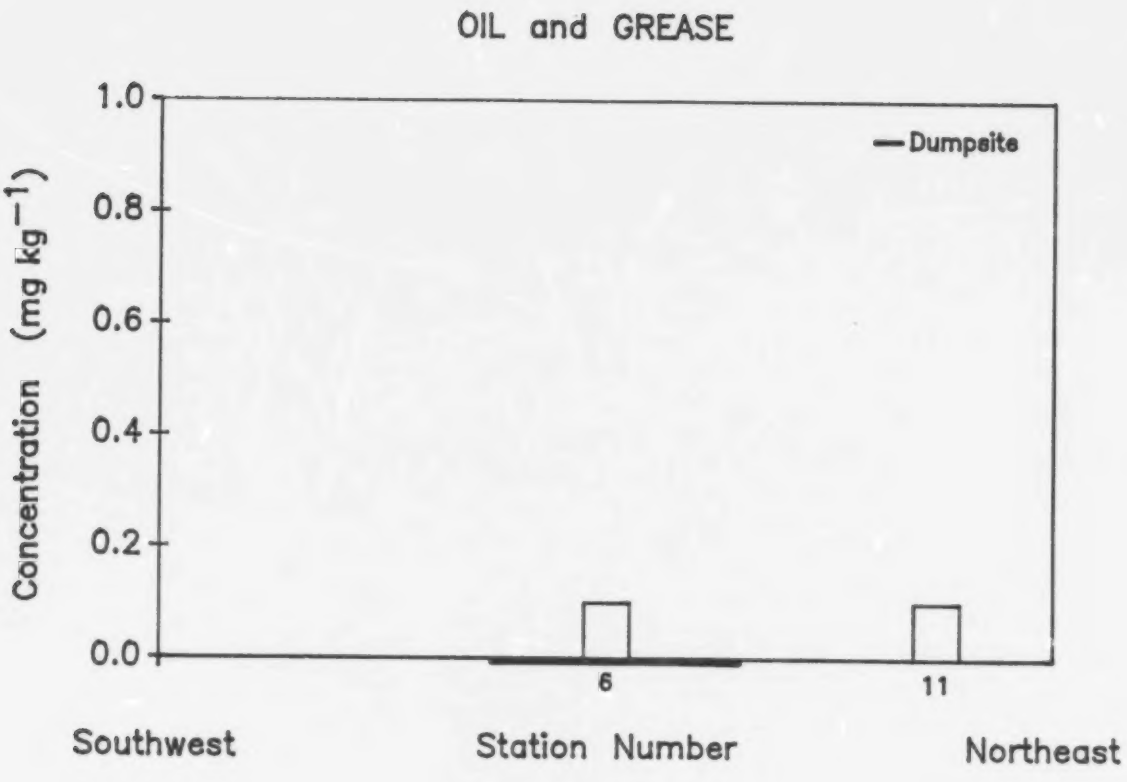


Figure 39: Sediment total oil and grease concentrations along a southwest-northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

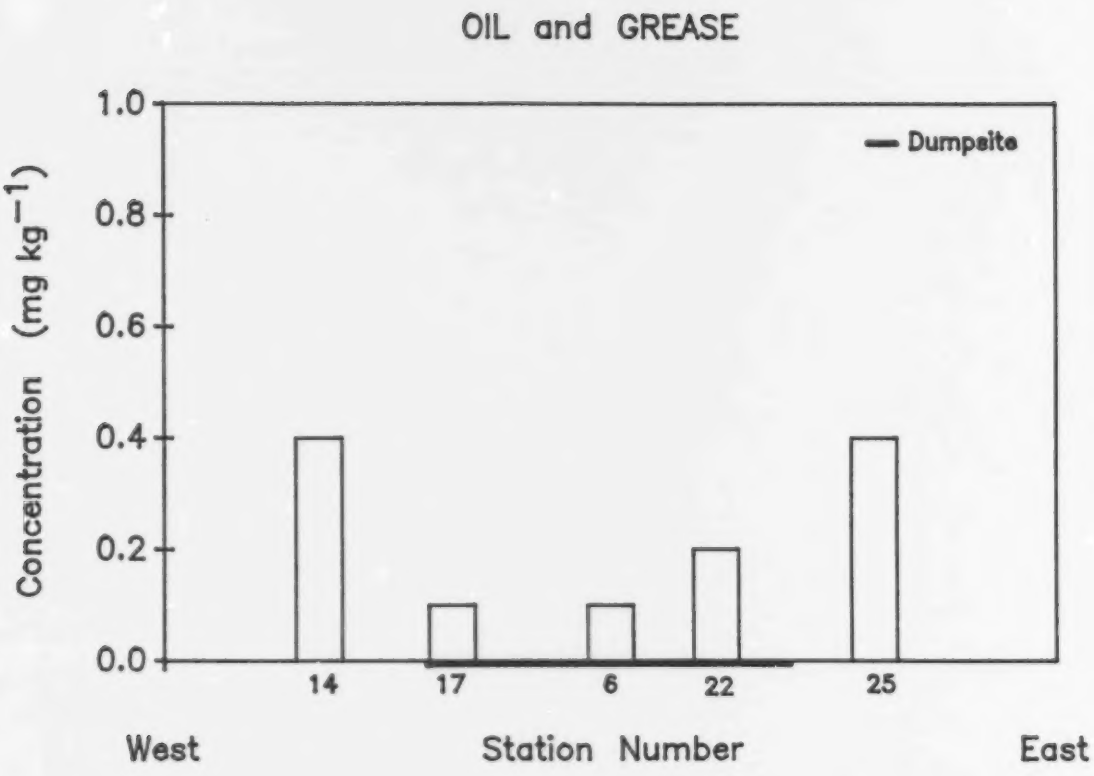


Figure 40: Sediment total oil and grease concentrations along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

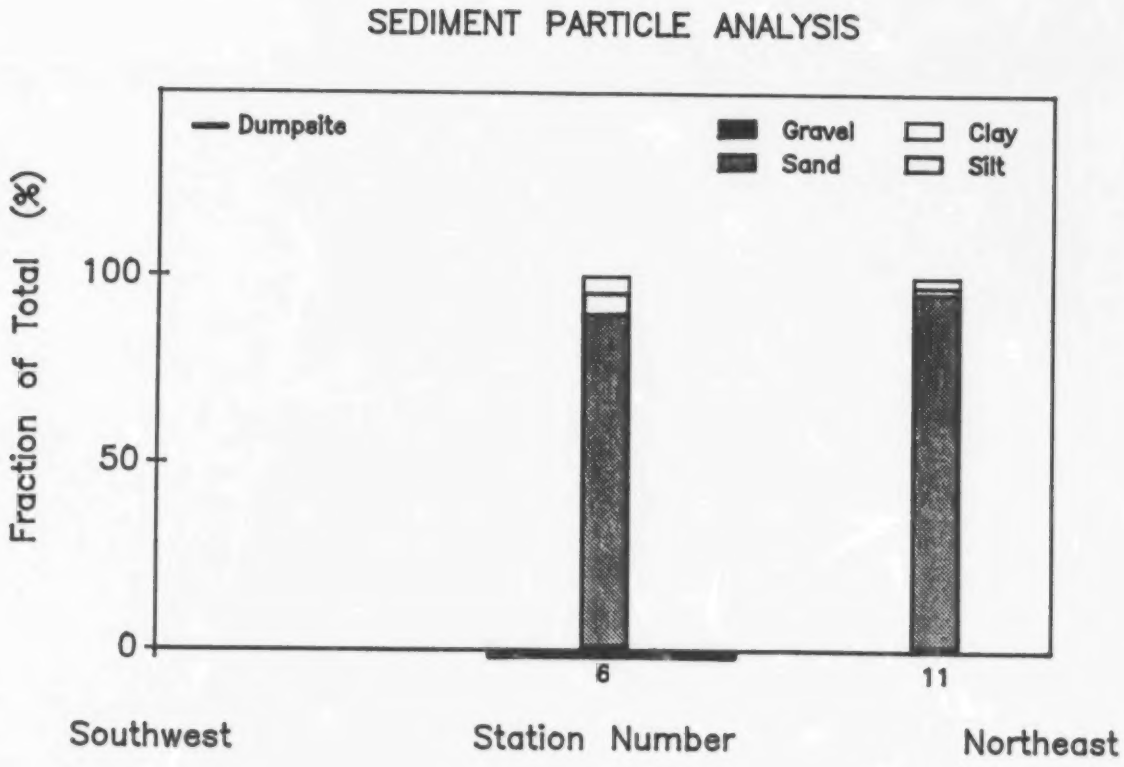


Figure 41: Sediment particle size fractions along a southwest- northeast transect through sediment dumpsite "A" in the Miramichi River, NB.

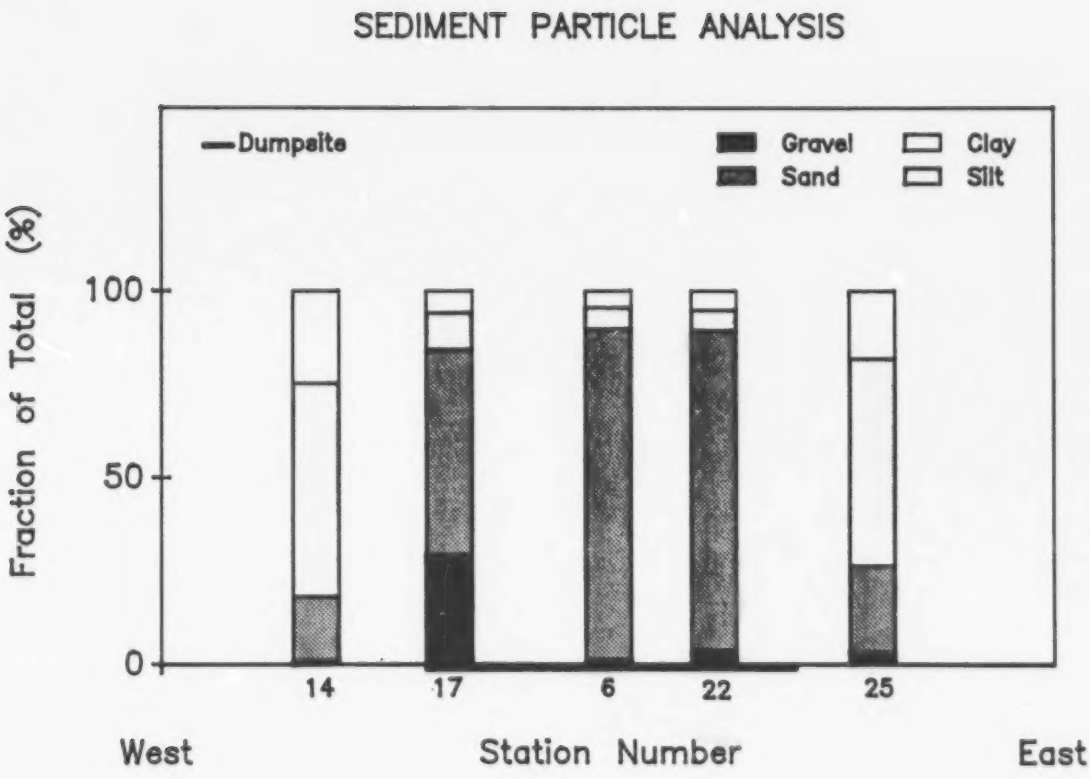


Figure 42: Sediment particle size fractions along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

A2. TABLES

Table 1: Sediment microbial exoenzyme activity in Pictou Harbour, Nova Scotia, July 7, 1989.

	Activity (uM g^{-1} h^{-1})					
Station	GLU	S.E.	PO4	S.E.	LEU	S.E.
1	8.4	0.6	95	13	0.9	0.2
2	46	6	233	32	22	3
3	14	2	102	11	1.2	0.2
4	24	2	104	13	8.2	0.9
5	4.0	0.4	22	3	1.0	0.1
6	6.5	0.6	24	0.8	1.9	0.2
7	10.6	0.5	68	4	2.4	0.3
8	19	2	82	10	1.9	0.2
9	19	3	109	5	3.5	0.4
11	25	1	146	13	2.5	0.3
12	3.9	0.3	17	2	0.88	0.09
13A	8	1	61	10	0.69	0.04
13B	6.0	0.4	49	4	0.50	0.05
14	14	1	82	3	2.2	0.2
15	18	3	82	5	1.2	0.1
16	15.6	0.8	70	4	1.5	0.1
17A	6.9	0.3	55	3	0.56	0.04
17B	13	1	80	4	1.3	0.2
18	6.4	0.4	53	6	0.39	0.04
19	7.5	0.3	46	4	0.34	0.04
20	20	3	116	15	2.2	0.2
21	7.0	0.4	46	3	0.59	0.07
22	12.9	0.9	70	7	0.77	0.07
23	7.2	0.2	76	4	0.41	0.04
24	12	1	70	8	1.0	0.1
25	13	3	64	3	0.9	0.1
26	17	2	82	6	1.5	0.2
27	15	1	163	15	1.08	0.09

GLU = Glucosidase
PO4 = Phosphatase
LEU = Protease
S.E. = Standard Error; n = 5

Table 2: Pearson correlation matrix of Pictou Harbour exoenzyme activity, water depth, and distance from dumpsite centre data (n = 28).

	Depth	GLU	PO4	LEU	Distance
Depth	1.000				
GLU	0.064	1.000			
PO4	-0.175	0.880*	1.000		
LEU	0.096	0.849*	0.704*	1.000	
Distance	0.134	0.327	0.345	0.266	1.000

GLU = Glucosidase
PO4 = Phosphatase
LEU = Protease

* Significant correlation (P<0.05)

Table 3: Pearson correlation matrix of all enzyme, physical and chemical data from Pictou Harbour, Nova Scotia (n = 10).

	DIST	DEPTH	GLU	PO4	LEU	TIC	TOC	TC	GRAV	SAND	SILT	CLAY	CD	CU	PB	ZN	OIL	PCB
DIST	1.000																	
DEPTH	-0.293	1.000																
GLU	0.244	-0.013	1.000															
PO4	0.361	-0.219	0.840*	1.000														
LEU	0.297	0.092	0.674*	0.554	1.000													
TIC	0.368	-0.581	0.493	0.456	0.361	1.000												
TOC	0.277	-0.525	0.094	0.235	0.093	0.806*	1.000											
TC	0.330	-0.581	0.304	0.361	0.236	0.948*	0.952*	1.000										
GRAV	-0.051	0.383	-0.114	-0.062	0.221	-0.214	0.032	-0.093	1.000									
SAND	-0.158	0.428	-0.437	-0.435	-0.488	-0.885*	-0.855*	-0.915*	-0.080	1.000								
SILT	0.215	0.181	0.474	0.178	0.399	0.364	0.212	0.301	-0.206	-0.414	1.000							
CLAY	0.039	-0.580	0.186	0.363	0.282	0.741*	0.797*	0.810*	0.205	-0.831*	-0.162	1.000						
CD	0.250	-0.186	0.115	0.224	0.430	0.561	0.776*	0.706*	0.093	-0.781*	0.492	0.542	1.000					
CU	0.119	-0.429	0.130	0.133	0.316	0.781*	0.882*	0.876*	0.152	-0.867*	0.118	0.867*	0.707*	1.000				
PB	0.441	-0.724	0.403	0.442	0.079	0.546	0.354	0.472	-0.564	-0.278	-0.005	0.310	0.040	0.317	1.000			
ZN	0.015	0.101	0.052	0.209	-0.121	0.203	0.578	0.415	0.318	-0.324	-0.075	0.392	0.377	0.479	0.094	1.000		
OIL	-0.437	-0.107	-0.173	-0.251	0.334	-0.164	-0.208	-0.196	0.070	-0.028	-0.238	0.175	0.034	0.159	-0.076	-0.407	1.000	
PCB	-0.193	-0.237	-0.418	-0.306	-0.208	0.320	0.592	0.483	0.433	-0.499	-0.084	0.590	0.417	0.545	-0.357	0.218	0.020	1.000

"*" Denotes significant correlation ($P<0.05$).

DIST = distance from dumpsite centre (m), DEPTH = water depth, GLU = glucosidase activity, PO4 = phosphatase activity, LEU = protease activity, TIC = total inorganic carbon concentration, TOC = total organic carbon concentration, TC = total carbon concentration, GRAV = gravel fraction proportion, SAND = sand fraction proportion, SILT = silt fraction proportion, CLAY = clay fraction proportion, CD = cadmium concentration, CU = copper concentration, PB = lead concentration, ZN = zinc concentration, OIL = oil and grease concentration, PCB = PCB concentration.

Table 4: Results of stepwise multiple regressions of Pictou Harbour, Nova Scotia, enzyme activity data ($p < 0.05$; $n = 28$).

Independant Variable	Dependant Variables	r^2 Value
GLU	PO4	0.775
	LEU	0.879
PO4	GLU	0.775
	DEPTH	0.829
LEU	GLU	0.721

GLU = Glucosidase activity
PO4 = Phosphatase activity
LEU = Protease activity
DEPTH = Water depth

Table 5: Sediment microbial exoenzyme activity in the Miramichi River, New Brunswick, July 9, 1989.

	Activity ($uM\ g^{-1}\ h^{-1}$)					
Station	**GLU**	**S.E.**	**PO4**	**S.E.**	**LEU**	**S.E.**
1	19	2	37	3	2.8	0.2
2	19	1	42	4	3.6	0.2
3	21	3	49	5	5.1	0.6
4	30	2	66	5	7	1
5	38	2	80	8	11	1
6A	7.5	0.7	17.1	0.8	3.1	0.2
6B	36	3	87	10	6.0	0.3
7	27	3	60	6	6	1
8	91	12	180	21	7.8	0.7
9	26	3	52	4	3.6	0.3
10	11	0.7	73	4	4.1	0.9
11	7.4	0.6	26	1	5.4	0.2
13A	54	3	129	13	5.1	0.4
13B	52	6	105	10	7	1
14	57	5	110	5	3.5	0.4
15	55	7	166	17	2.5	0.2
16	45	3	80	11	12	1
17	17	1	32	2	4.9	0.8
18	36	2	58	4	9.1	0.7
19	37	4	47	3	5.0	0.3
20	19	3	65	5	1.7	0.2
21	14	1	42	4	4.5	0.4
22	34	2	96	5	10.7	0.4
23	10.9	0.4	45	4	9.3	0.9
25	25	4	42	3	3.5	0.7

GLU = Glucosidase
PO4 = Phosphatase
LEU = Protease
S.E. = Standard Error; n = 5

Table 6: Pearson correlation matrix of Miramichi River exoenzyme activity, water depth, and distance from dumpsite centre data (n = 25).

	Depth	GLU	PO4	LEU	Distance
Depth	1.000				
GLU	-0.020	1.000			
PO4	-0.138	0.895*	1.000		
LEU	-0.003	0.275	0.172	1.000	
Distance	-0.305	0.079	0.115	-0.219	1.000

GLU = Glucosidase
PO4 = Phosphatase
LEU = Protease

* Significant correlation (P<0.05)

Table 7: Pearson correlation matrix of all enzyme, physical and chemical data from the Miramichi River, New Brunswick, Dumpsite A (n=6).

	DIST	DEPTH	GLU	PO4	LEU	TIC	TOC	TC	GRAV	SAND	SILT	CLAY	CD	CU	PB	ZN	OIL	PCB
DIST	1.000																	
DEPTH	0.209	1.000																
GLU	0.299	-0.546	1.000															
PO4	0.204	-0.656	0.945*	1.000														
LEU	-0.184	-0.515	0.123	0.421	1.000													
TIC	0.488	-0.385	0.812*	0.609	-0.348	1.000												
TOC	0.472	-0.388	0.870*	0.687	-0.312	0.986*	1.000											
TC	0.476	-0.388	0.861*	0.674	-0.319	0.991*	1.000*	1.000										
GRAV	-0.145	0.450	-0.196	-0.252	0.022	-0.252	-0.311	-0.301	1.000									
SAND	-0.448	0.186	-0.673	-0.417	0.456	-0.932*	-0.880*	-0.892*	-0.084	1.000								
SILT	0.488	-0.322	0.685	0.451	-0.461	0.981*	0.941*	0.950*	-0.234	-0.948*	1.000							
CLAY	0.464	-0.324	0.801	0.581	-0.417	0.995*	0.987*	0.991*	-0.238	-0.935*	0.976*	1.000						
CD	0.243	-0.255	0.846*	0.680	-0.362	0.831*	0.902*	0.891*	-0.312	-0.702	0.749	0.868*	1.000					
CU	0.443	-0.413	0.919*	0.758	-0.234	0.967*	0.993*	0.991*	-0.283	-0.854*	0.901*	0.968*	0.919*	1.000				
PB	0.265	-0.160	0.787	0.606	-0.443	0.803	0.876*	0.864*	-0.323	-0.679	0.730	0.847*	0.993*	0.887	1.000			
ZN	0.433	-0.346	0.928*	0.788	-0.218	0.903*	0.961*	0.952*	-0.327	-0.758	0.813*	0.915*	0.960*	0.980*	0.938*	1.000		
OIL	0.464	-0.550	0.768	0.609	-0.217	0.968*	0.939*	0.946*	-0.365	-0.864*	0.959*	0.941*	0.717	0.912*	0.675	0.824*	1.000	
PCB	0.161	0.364	0.204	0.045	-0.187	0.224	0.173	0.183	0.876*	-0.520	0.223	0.244	0.130	0.195	0.116	0.144	0.069	1.000

"*" Denotes significant correlation (P<0.05).

DIST = distance from dumpsite centre (m), DEPTH = water depth, GLU = glucosidase activity, PO4 = phosphatase activity, LEU = protease activity, TIC = total inorganic carbon concentration, TOC = total organic carbon concentration, TC = total carbon concentration, GRAV = gravel fraction proportion, SAND = sand fraction proportion, SILT = silt fraction proportion, CLAY = clay fraction proportion, CD = cadmium concentration, CU = copper concentration, PB = lead concentration, ZN = zinc concentration, OIL = oil and grease concentration, PCB = PCB concentration.

Table 8: Results of stepwise multiple regressions of Miramichi River, New Brunswick, enzyme activity data ($p < 0.05$; $n = 25$).

Independant Variable	Dependant Variables	r^2 Value
GLU	PO4	0.801

GLU = Glucosidase activity
PO4 = Phosphatase activity

Table 9: Dumpsite characteristics and history.

Dumpsite	Physical/Chemical	Quantity (m^3)	Reference
Pictou Hbr. Dumpsite 1988	Mostly organic clay; PCB's up to 800ppb in some surface samples of dredged materials dumped at the site.	195,000	OceanChem Group, 1987
Miramichi Dumpsite A 1981-83	Mostly silt and clay; Some sediment contained trace levels of Cd.	1,446,500	MacLaren Plansearch Ltd., 1985
Dalhousie Heron Island Dumpsite 1973-77	Mostly silty clay, wood chips and Zn/Cd ores.	866,000	Packman et al., 1984

A3. CHEMICAL DATA

Table 1: Sand, Silt, Clay
Total Organic/Inorganic Carbon Pictou - Miramichi

OCL #	Client I.D.	% Gravel	% Sand	% Silt	% Clay	TIC g/kg	TOC g/kg	TC g/kg
3280	Pictou Hbr. P1	0.0	3.0	50.7	46.2	54.5	56.4	110.9
3281	Pictou Hbr. P3	0.1	6.4	53.9	39.5	46.0	53.2	99.2
3282	Pictou Hbr. P5	0.0	8.5	58.6	32.9	42.9	46.0	88.9
3283	Pictou Hbr. P7	0.2	11.5	51.5	36.8	39.6	39.5	79.1
3284	Pictou Hbr. P8	0.0	4.6	58.2	37.3	56.4	47.4	10.38
3285	Pictou Hbr. P9	0.0	6.2	57.7	36.0	48.6	45.2	93.8
3286	Pictou Hbr. P11	0.0	6.9	57.2	35.9	47.5	41.0	88.5
3287	Pictou Hbr. P13	0.0	22.0	43.2	34.8	37.6	35.0	72.6
3288	Pictou Hbr. P15	0.0	12.5	58.6	29.0	44.1	40.6	84.7
3289	Pictou Hbr. P17	0.0	30.5	53.9	15.7	32.2	31.3	63.5
3290	Miramichi M6	1.4	88.5	5.7	4.4	11.3	10.1	21.4
3291	Miramichi M11	1.4	94.4	1.8	2.5	10.7	7.2	17.9
3292	Miramichi M14	1.2	17.0	57.1	24.7	31.0	99.6	130.6
3293	Miramichi M17	29.5	55.0	9.8	5.8	13.3	12.4	25.7
3294	Miramichi M22	3.8	85.6	5.5	5.1	14.2	23.2	37.4
3295	Miramichi M25	3.3	23.3	55.4	18.1	26.2	61.5	87.7

Table 2: Trace Metal Results Pictou - Miramichi
Concentration mg/kg

OCL #	Client Identification	Cadmium	Copper	Lead	Zinc
3280A	Pictou Hbr. P1	0.45	30.7	54.3	179
3280B	Pictou Hbr. P1	0.43	29.9	50.2	169
3281	Pictou Hbr. P3	0.46	30.4	43.6	166
3282	Pictou Hbr. P5	0.47	28.1	33.3	129
3283	Pictou Hbr. P7	0.40	27.0	31.1	123
3284	Pictou Hbr. P8	0.40	29.9	47.0	149
3285	Pictou Hbr. P9	0.47	29.9	52.8	145
3286A	Pictou Hbr. P11	0.42	24.4	43.3	128
3286B	Pictou Hbr. P11	0.41	25.9	45.7	123
3287	Pictou Hbr. P13	0.32	25.3	46.7	144
3288	Pictou Hbr. P15	0.37	25.4	51.8	127
3289	Pictou Hbr. P17	0.35	19.2	39.2	97
3290	Miramichi M6	0.32	4.7	24.8	58
3291	Miramichi M11	0.94	4.2	17.3	57
3292	Miramichi M14	0.99	21.6	47.2	197
3293	Miramichi M17	0.17	5.8	18.5	57
3294	Miramichi M22	0.23	8.4	18.3	82
3295A	Miramichi M25	0.33	13.1	23.7	100
3295B	Miramichi M25	0.35	13.6	25.8	99

OceanChemGroup

Table 3: Oil and Grease and PCB Results Pictou - Miramichi

OCL #	Client	Oil & Grease mg/kg	PCB (ng/g)
3280	Pictou Hbr. P1	0.3	101
3281	Pictou Hbr. P3	0.1	76
3282	Pictou Hbr. P5	0.6	100
3283	Pictou Hbr. P7	0.6	96
3284	Pictou Hbr. P8	0.2	72
3285	Pictou Hbr. P9	0.7	28
3286	Pictou Hbr. P11	0.3	48
3287	Pictou Hbr. P13	0.7	49
3288	Pictou Hbr. P15	0.4	47
3289	Pictou Hbr. P17	0.1	30
3290	Miramichi M6	0.1	11
3291	Miramichi M11	<0.1	19
3292	Miramichi M14	0.4	66
3293	Miramichi M17	0.1	127
3294	Miramichi M22	0.2	23
3295	Miramichi M25	0.4	48

Table 4: Quality Assurance Results

PCB		(publ.)
HS-2 - Reference Material	126	112 + 10%
Replicate P 13	50	47
Oil & Grease	<0.1	<0.1
Replicate	-	-
Metals -		
BCSS-1 (Ref. Material)		(publ.)
Cadmium	0.25	0.25 + 0.04
Copper	17.9	18.5 + 2.7
Zinc	119.	119 + 12
Lead	26.6	22.7 + 3.4
MESS-1 (Ref. Material)		(publ.)
Cadmium	0.62	0.59 + 0.10
Copper	24.2	25.1 + 3.8
Zinc	187.	191 + 17
Lead	34.9	34.0 + 6.1
Replicate Cadmium		
P1	0.45	0.43
P111	0.47	0.42
M25	0.33	0.34
Replicate Copper		
P1	30.7	29.9
P11	29.9	24.4
M25	13.1	13.6
Replicate Lead		
P1	54.3	50.2
P11	43.3	45.7
M25	23.7	25.8
Replicate Zinc		
P1	179	169
P11	128	123
M25	100	99

A4. SAMPLING STATION LOCATIONS

Pictou Harbour

In Pictou Harbour sampling stations were located approximately 200 - 220 meters apart, along four transects through two sediment dumpsites (Fig. 1).

Stations:	1 -> 8	bearing 222° from the light on Pictou Pier C.
	9 -> 17	bearing 65° from the light on Pictou Pier C.
	18 -> 21	bearing 152° from the triangulation marker at Campbell Point.
	22 -> 25	bearing 301.5° from the triangulation marker at Mussel Point.

Station Locations:

Stn	Latitude	Longitude	Depth (m)
1	45°39.79'N	62°43.07'W	8
2	45°39.88'N	62°42.96'W	10.5
3	45°39.96'N	62°42.85'W	13
4	45°40.05'N	62°42.74'W	13
5	45°40.15'N	62°42.62'W	12.5
6	45°40.24'N	62°42.50'W	13
7	45°40.33'N	62°42.39'W	13
8	45°40.41'N	62°42.29'W	12
9	45°40.48'N	62°42.11'W	11.5
11	45°40.58'N	62°41.80'W	12
12	45°40.64'N	62°41.66'W	12.5

Stn	Latitude	Longitude	Depth (m)
13	45°40.68'N	62°41.52'W	11.5
14	45°40.73'N	62°41.37'W	11
15	45°40.77'N	62°41.25'W	11
16	45°40.82'N	62°41.10'W	11.5
17	45°40.86'N	62°40.96'W	13.5
18	45°40.77'N	62°41.59'W	6.5
19	45°40.69'N	62°41.53'W	11.5
20	45°40.64'N	62°41.48'W	11
21	45°40.58'N	62°41.44'W	8
22	45°40.52'N	62°41.39'W	6
23	45°40.21'N	62°42.78'W	6
24	45°40.18'N	62°42.71'W	11
25	45°40.16'N	62°42.63'W	12.5
26	45°40.12'N	62°42.56'W	9
27	45°40.10'N	62°42.51'W	5.5

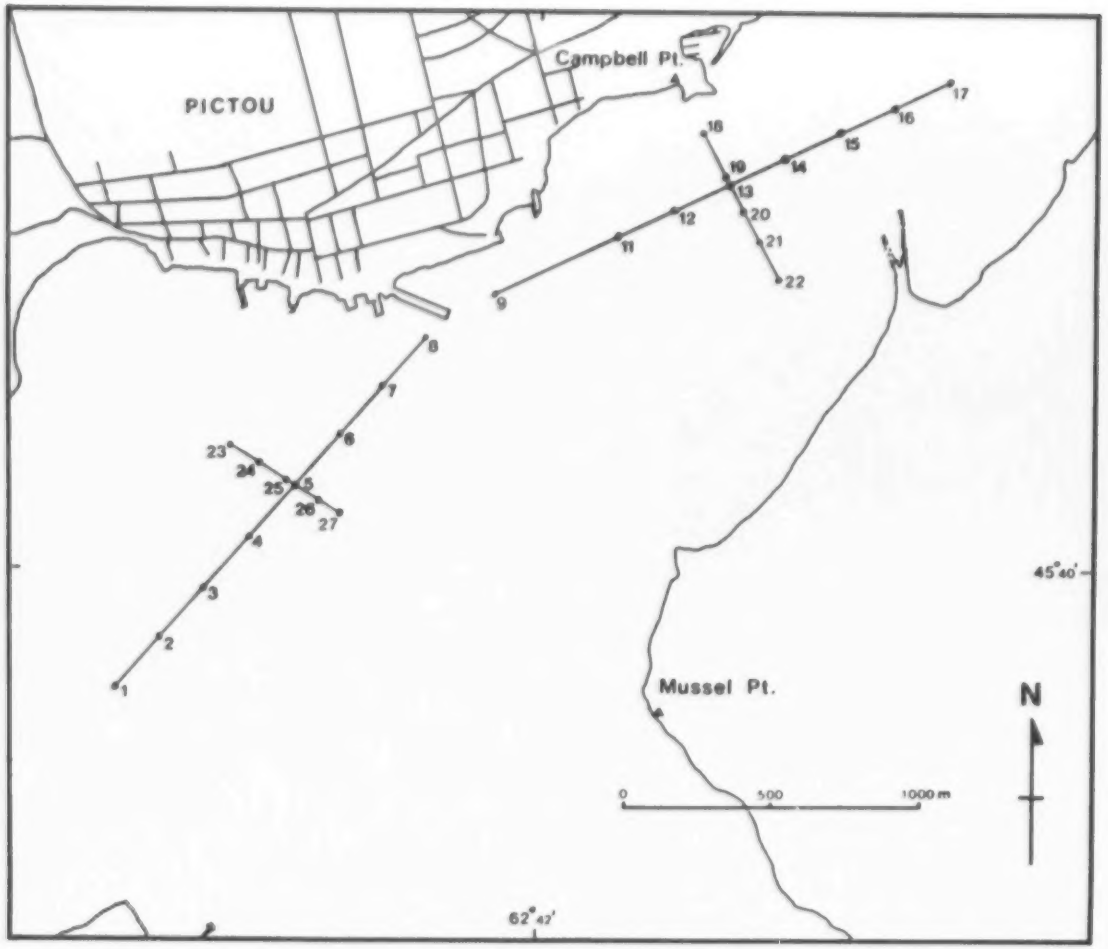


Figure 1: Sampling stations and locations of sediment dumpsites (shaded areas) in Pictou Harbour, Nova Scotia.

Miramichi River

Sampling stations (approximately 200 - 220 meters apart) in the Miramichi River lay on two transects through the sediment dumpsite. These transects, one in an east/west direction and the other in a northeast/southwest direction across the dumpsite, were positioned so as to pass through both the dumpsite centre and a convenient navigation marker.

Stations: 1 -> 12 bearing 76° from the lighted red buoy M90.

13 -> 25 bearing 210° from the chimney northeast of Morrison Cove.

Station Locations:

Stn	Latitude	Longitude	Depth (m)
1	47°00.93'N	65°31.65'W	5
2	47°00.99'N	65°31.47'W	4
3	47°01.05'N	65°31.30'W	4
4	47°01.11'N	65°30.78'W	5
5	47°01.15'N	65°30.56'W	5.5
6	47°01.18'N	65°30.33'W	6
7	47°01.22'N	65°30.10'W	6
8	47°01.26'N	65°29.86'W	5
9	47°01.30'N	65°29.63'W	5
10	47°01.34'N	65°29.42'W	4
11	47°01.38'N	65°29.18'W	7
13	47°01.18'N	65°31.47'W	5
14	47°01.18'N	65°31.38'W	5.5
15	47°01.18'N	65°31.28'W	6
16	47°01.18'N	65°31.09'W	6

Stn	Latitude	Longitude	Depth (m)
17	47°01.18'N	65°30.90'W	7
18	47°01.18'N	65°30.71'W	6.5
19	47°01.18'N	65°30.52'W	6
20	47°01.18'N	65°30.29'W	6
21	47°01.18'N	65°30.14'W	5
22	47°01.18'N	65°29.95'W	4
23	47°01.18'N	65°29.76'W	5
25	47°01.18'N	65°29.38'W	5

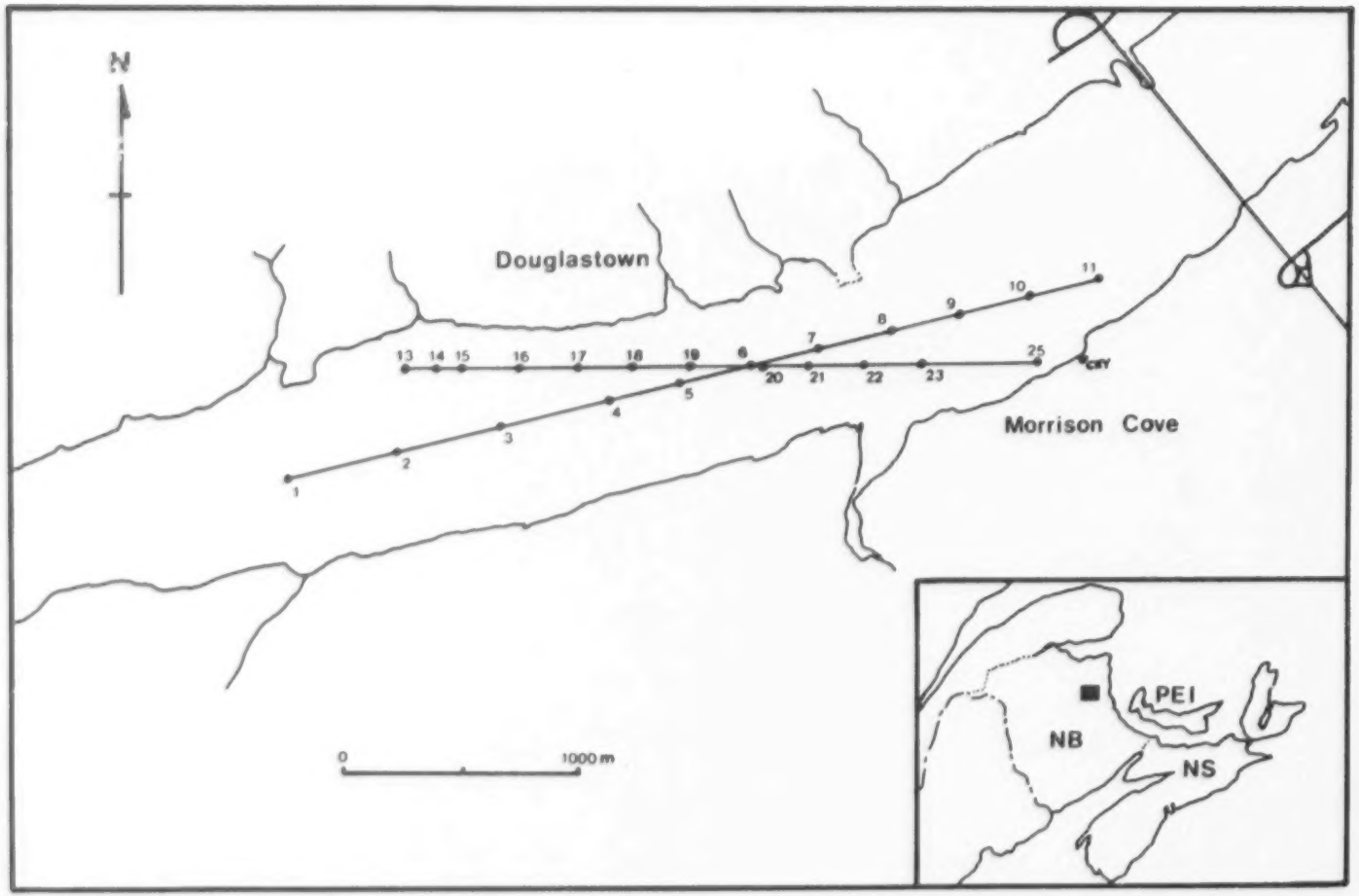


Figure 2: Sampling stations and location of sediment dumpsite "A" (shaded area) in the Miramichi River, New Brunswick.

A5. PROCEDURE FOR THE MEASUREMENT OF SEDIMENT EXOENZYME ACTIVITY

Introduction

Since bacteria are unable to assimilate large macromolecules directly, extracellular enzymatic breakdown must occur before uptake. This breakdown is accomplished by exoenzymes induced by the presence of a suitable substrate. The activity of specific inducible exoenzymes can be measured through the use of substrate analogues conjugated with the highly fluorescent compound, methylumbelliferone (MUF).

In the following procedure, the enzyme specific substrates 4-methylumbelliferyl-B-D-glucoside (for glucosidase), 4- methylumbelliferyl-phosphate (for phosphatase), 4-methylumbelliferyl-cellobioside (for cellulase) and L-leucine-4-methylcoumarinyl-amide (for protease) are used to quantify enzyme activity in slurries of sediment suspended in sea water. Enzymatic hydrolysis of these MUF-substrates yields equimolar amounts of methylumbelliferone and product (eg. glucose in the case of MUF-glucoside). The high fluorescence of methylumbelliferone allows measurement of enzymatic activity at ambient temperatures after short incubation times, and at substrate concentrations similar to natural background levels.

Outline of method

Freshly collected sediment is suspended in 0.2 um filtered seawater and incubated in the presence of a 5 uM MUF-substrate. Following incubation, the slurry is frozen to arrest metabolic activity. For fluorescence analysis, the slurry is thawed and then centrifuged. The supernatant is decanted into a cuvette and a buffer is added to maximize the fluorescence of the liberated MUF. Enzyme activity is calculated from the amount of MUF liberated over the incubation period (Figure 1).

Reagents

Synthetic Ocean Water (SOW) is prepared according to Morel et al. (1979). To prepare 1 L of SOW, reagent grade salts are added to distilled water in the following order:

Salt	Amount (g)
NaCl	24.53
$CaCl_2.2H_2O$	1.54
KBr	0.10
NaF	0.003
KCl	0.70
H_3BO_3	0.03
Na_2SO_4	4.09
$NaHCO_3$	0.20
$SrCl_2.6H_2O$	0.017
$MgCl_2.6H_2O$	11.10

(The mixture is adjusted to pH 8.0 using 1 N $NaHCO_3$).

Methylumbelliferyl exoenzyme substrate analogue stock solutions (5mM) in a 10% (v/v) solution of methanol/SOW are prepared as follows:

For cellulase, phosphatase and glucosidase substrates; 62.6 mg of 4-methylumbelliferyl-B-D-cellobioside, 37.5 mg of 4-methylumbelliferyl phosphate or 42.3 mg of 4- methylumbelliferyl-B-D-glucoside (Sigma Chemical Co.) is dissolved in 2.5 mL of reagent grade methanol and diluted to a volume of 25 mL with SOW.

To prepare the protease substrate 16.2 mg of L-Leucine-4-methylcoumarinyl-7-amide HCl (Fluka Chemical Corp.) is dissolved in 1.0 mL of reagent grade methanol and diluted to a volume of 10 mL with SOW.

Stock solutions of MUF substrates are stored at -20°C when not in use. Working enzyme stocks are prepared by making 1:33 dilutions of the 5 mM stocks with SOW.

Methylumbelliferone Standard is prepared as a 2.5 mM solution in reagent grade methanol using 4-methylumbelliferone (Sigma Chemical Co.). A working standard solution (1 uM) is prepared by diluting 40 uL of the above standard in 100 mL of SOW.

NaOH/Borax Buffer (pH 10.8) is prepared by mixing 67 mL of 0.2 M NaOH with 50 mL of 0.05 M sodium tetraborate to a final volume of 200 mL with distilled water.

Activity measurement

Sediment microbial enzyme activity is measured in slurries of sediment using a modification of the technique described by Meyer-Reil (1986).

Sediment preparation

Replicate subsamples (5) of homogeneously mixed sediment approximately 0.2 cm^3 are dispensed into pre-weighed 7 mL polypropylene test tubes (CanLab/Travenol Canada Inc.) using micro core tubes (4 mm ID glass tubing) fitted with a Pasteur pipette bulb.

Assay procedure

Once dispensed into the assay tubes, the sediment is suspended in 0.2 um filtered seawater. The tubes are capped with polyethylene stoppers, agitated and placed in an incubator at *in situ* temperature for 30 minutes. After this pre-incubation period, specific 100 uL aliquots of 0.15 mM enzyme substrate solutions are added to each tube (final substrate concentration of 5 uM). The assay tubes are incubated for 60 minutes with agitation at 20 minute intervals. The tubes are frozen in dry ice to

arrest enzyme activity and transported to the laboratory for fluorescence analysis. Duplicate blanks (SOW and sediment) and standards (1 uM MUF and sediment) are run concurrently.

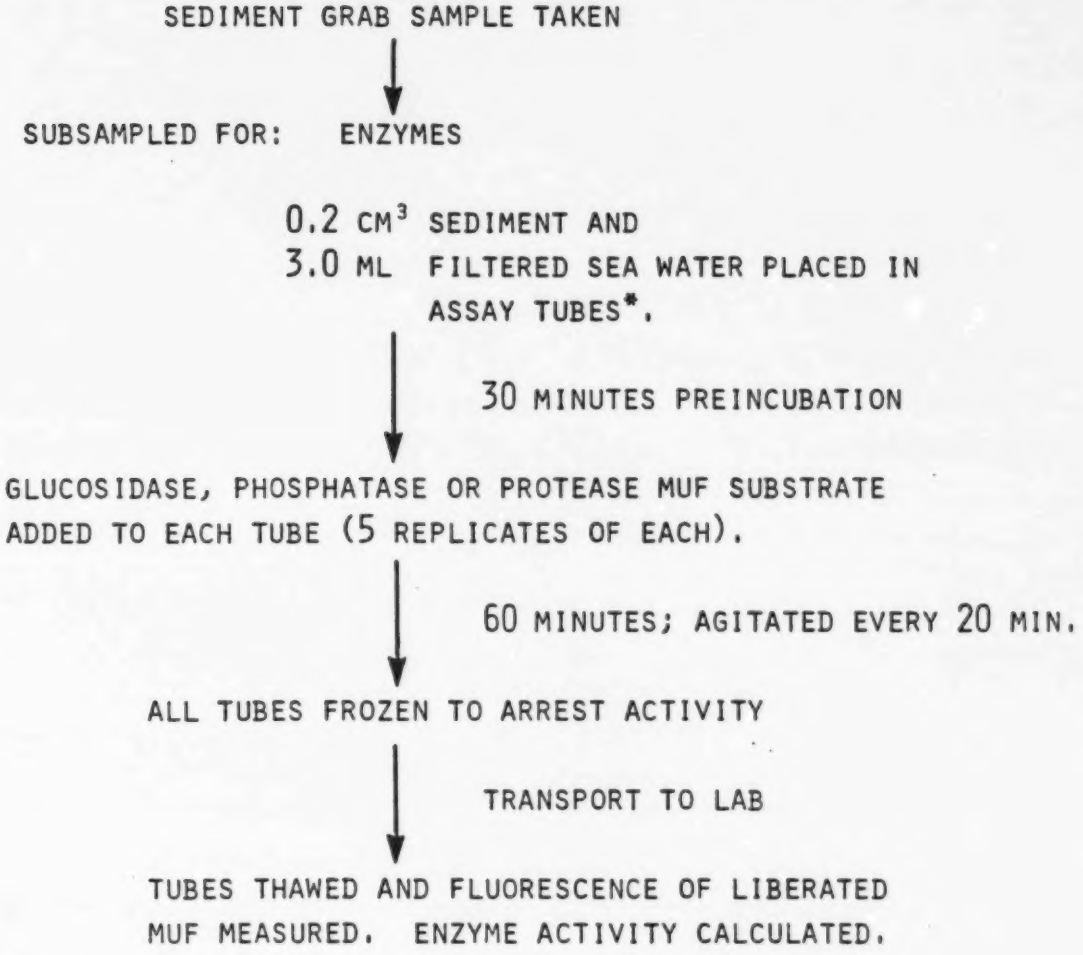
Fluorescence measurement

The fluorescence of liberated methylumbelliferone (MUF) is measured using a Perkin-Elmer MPF-44B fluorescence spectrophotometer. Tubes are thawed in an ice bath and centrifuged at 1450 rpm for 5 minutes. The supernatant (2.5 mL) is decanted into a 1 cm path-length cuvette and 0.25 mL of NaOH/Borax buffer is added to maximize the fluorescence of the liberated MUF. Fluorescence is measured at 455 nm emission with 365 nm excitation. The MUF concentration in each tube is calculated by comparison of the fluorescence to a standard curve prepared using known dilutions of a 1 uM MUF standard solution.

Calculation of results

After fluorescence analysis, experimental tubes with the remaining sediment were dried overnight to constant weight in a convection oven (90°C) to determine sediment dry weight. Exoenzyme activity is calculated as uM product/g sediment/h (+/- S.D.).

ENZYME ASSAY PROCEDURE


SEDIMENT GRAB SAMPLE TAKEN
SUBSAMPLED FOR: ENZYMES
0.2 CM3 SEDIMENT AND
3.0 ML FILTERED SEA WATER PLACED IN ASSAY TUBES*.
30 MINUTES PREINCUBATION
GLUCOSIDASE, PHOSPHATASE OR PROTEASE MUF SUBSTRATE ADDED TO EACH TUBE (5 REPLICATES OF EACH).
60 MINUTES; AGITATED EVERY 20 MIN.
ALL TUBES FROZEN TO ARREST ACTIVITY
TRANSPORT TO LAB
TUBES THAWED AND FLUORESCENCE OF LIBERATED MUF MEASURED. ENZYME ACTIVITY CALCULATED.

* 1.0 UM MUF STANDARD ADDED TO DUPLICATE TUBES TO PROVIDE INTERNAL STANDARDS.

Figure 1: Flow chart of sediment exoenzyme activity assay.

A6. SAMPLE CORRECTION FOR QUENCHING OF FLUORESCENCE

Introduction

The fluorescence of methylumbelliferone (MUF) standards containing Miramichi River sediments was very low compared to identical standards not containing sediments. Since enzyme activity calculations were based on the comparison of the concentrations of MUF liberated from enzyme specific substrates with standards of known concentration, this interference with MUF fluorescence would result in artificially low estimates of enzyme activity. This interference was dependant on the concentration of sediment present in the assay tubes. Since the magnitude of the interference varied with the sediment source (Fig. 1), a single correction factor for all samples could not be employed. Thus, a sample specific method of interference correction was developed, based on the fluorescence of the internal standards (MUF standard solutions containing sediments) that were run concurrently with the enzyme assays.

Interference correction

The fluorescence of the 1.0 uM MUF internal standard tubes, containing sediment, was measured and compared to standard solution without sediment. A correction factor was calculated as;

$$Z = \frac{\text{fluor. of std. without sed.}}{\text{fluor. of std. with sed.}}$$

The MUF concentration in each enzyme assay tube was then calculated by comparing the fluorescence in each tube with a standard curve prepared with standard solutions exposed to sediment. The resulting data were corrected for sediment interference by multiplying the data by the correction factor "Z" and the enzyme activity was calculated.

Verification of correction factors

In order to verify the utility of this internal standard correction factor, the results were compared with a more rigorous, and labour intensive, assay tube specific correction method. In this method MUF standards were incubated with a range of sediment concentrations and the MUF fluorescence measured by comparison to external standards without sediment (Fig. 1). A curvilinear regression of the data from station 25 yielded the following equation;

$$Y = (-1.059 /X + 1.006)^2 \qquad r = 0.989$$

(X = sediment dry weight; Y = fluorescence of 1.0 uM standard)

Thus, the measured MUF concentration in each tube was corrected based on the amount of sediment present in that tube using the above equation. Enzyme activity was then calculated using the corrected MUF concentration.

Since there was little variation in the amount of sediment dispensed into each assay tube (mean = 0.217 $\pm$ 0.065 g for Stn 25), enzyme activity data calculated using both methods were not significantly different (Table 1). Thus it was concluded that a single correction factor could be calculated for each station employing the internal standards run concurrently with the enzyme assays.

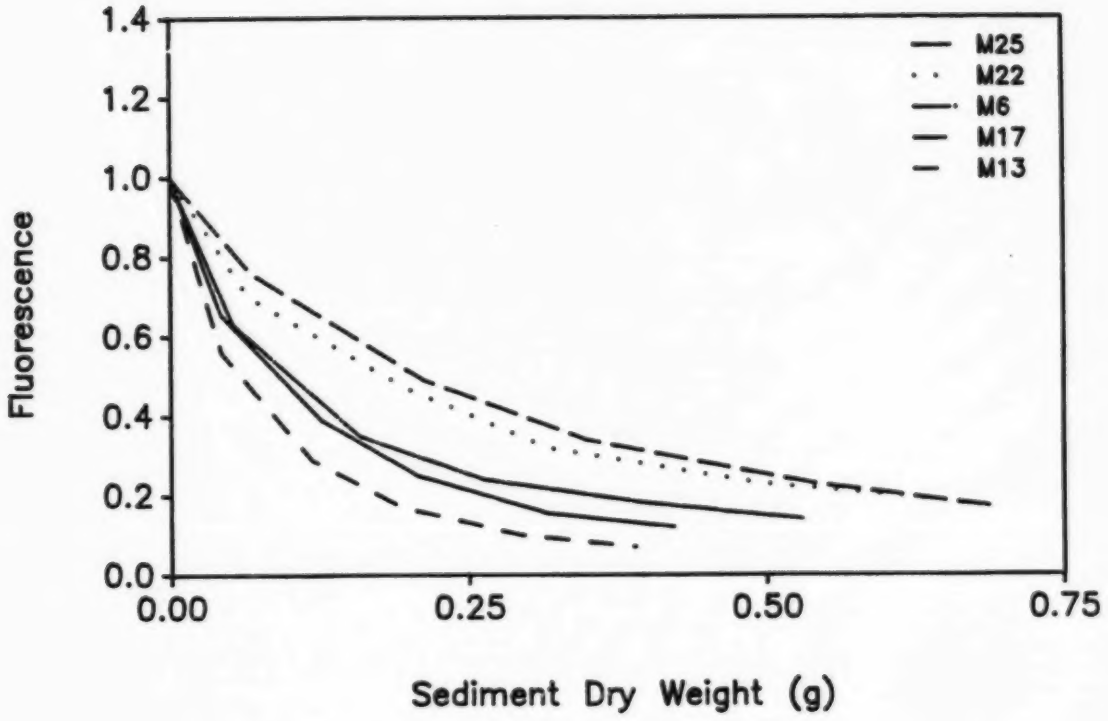


Figure 1: Fluorescence of 1.0 uM methylumbelliferone standard after incubation with different concentrations of sediment sampled along a east-west transect through sediment dumpsite "A" in the Miramichi River, NB.

Table 1: Comparison of enzyme activity (uM g^{-1} h^{-1}; mean $\pm$ s.d.) at station 25 in the Miramichi River, calculated using correction factors derived from external and internal standards.

	STANDARDS	
ENZYME	INTERNAL	EXTERNAL
Glucosidase	24.9 $\pm$ 9.4	36.2 $\pm$ 3.4
Phosphatase	42.1 $\pm$ 7.1	48.1 $\pm$ 4.7
Protease	3.5 $\pm$ 1.5	4.1 $\pm$ 1.2